COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY OF TRIPHALA, TERMINALIA CHEBULA & CHLORHEXIDINE ON ENTEROCOCCUS FAECALIS – AN INVITRO STUDY

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CERTIFICATE

This is to certify that **Dr. S. Jothi Latha**, post graduate student (2007 – 2010) in the Department of Conservative Dentistry and Endodontics, Tamil Nadu Government Dental College and Hospital, Chennai-3, has done this dissertation titled "**Comparative evaluation of antimicrobial activity of Triphala, Terminalia chebula & Chlorhexidine on Enterococcus faecalis – An InVitro study**" under our direct guidance and supervision in partial fulfillment of the regulations laid down by The Tamil Nadu Dr. M.G.R. Medical University, Guindy, Chennai-32 for M.D.S. – Conservative Dentistry and Endodontics (Branch IV) Degree Examination.

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INTRODUCTION

The success of endodontic treatment is directly influenced by elimination of microorganisms in infected root canals.[11] Microorganisms that invade the root canal system have an essential role in initiating and sustaining periapical disease.[47]

Unlike primary endodontic infections, which are polymicrobial in nature and dominated by gram-negative anaerobic rods, the microorganisms involved in secondary infections are composed of one or a few bacterial species.[60, 85, 36] Enterococcus faecalis is a persistent organism that, despite making up a small proportion of the flora in untreated canals, plays a major role in the etiology of persistent periradicular lesions after root canal treatment. It is commonly found in a high percentage of root canal failures and it is able to survive in the root canal as a single organism or as a major component of the flora.[22] Studies investigating its occurrence in root-filled teeth with periradicular lesions have demonstrated a prevalence ranging from 24 to 77%.[36, 60, 67, 68, 70, 71, 78, 85] Starvation increases the resistance of E. faecalis 1000-fold to 10,000-fold. It is probable that the physiologic state of the cells, particularly in retreatment cases, is closest to the starvation phase.[72]

The majority of infecting bacteria, together with their principal substrate of necrotic pulp debris, might be reduced significantly by chemomechanical instrumentation.[27, 57] However, this is not always achieved completely as a result of the anatomic complexity and diversity of root canals, as well as the subsequent limitations in access by instruments and irrigants.[46, 69, 82] Therefore, the use of an intracanal medication can help eliminate the bacteria remaining even after chemomechanical instrumentation and can provide an environment conducive to periapical tissue repair.[16]

Calcium hydroxide has been used as an intracanal medicament for a long time. The effects of calcium hydroxide [$Ca(OH)_2$] in root canal and dentin disinfection are well documented. The antimicrobial effects of $Ca(OH)_2$ may be directly related to its high alkalinity and a great majority of the microbial species isolated from root canals show susceptibility both clinically and ex vivo (Bystrom & Sundqvist 1981, 1983, Bystrom et al. 1985, Waltimo et al. 2005). However, several studies demonstrated that $Ca(OH)_2$ fails to eradicate Enterococcus faecalis residing in infected root canal systems (Bystrom et al. 1985, Haapasalo & Ørstavik 1987, Safavi et al. 1990, Ørstavik & Haapasalo 1990, Evans et al. 2002, Peters et al. 2002, Weiger et al. 2002, Saleh et al. 2004). An explanation for the resistance

against $Ca(OH)_2$ might be the ability of E. faecalis to invade dentinal tubules, isthmuses and other ramifications of a root canal system (Love 2001). Furthermore, it has been documented to be able to survive for prolonged periods in high alkalinity (Bystrom et al. 1985, Haapasalo & Ørstavik 1987) and harsh nutrient conditions (Hartke et al. 1998, Figdor et al. 2003, Portenier et al. 2005). Evans et al. (2002) reported the survival of E. faecalis at high pH was due to a functioning proton pump with the capacity to acidify cytoplasm. On the other hand, the buffering capacity of dentin can inhibit the pH increase and the antimicrobial activity of $Ca(OH)_2$ in the root canal (Haapasalo et al. 2000).

Many alternative antimicrobial agents have been tested for their ability to eliminate E. faecalis from the root canal system. In vitro studies have indicated that chlorhexidine may be potent in the elimination of E. faecelis from the root canal system (Bystrom et al.1985, Haapasalo & Ørstavik 1987, Ørstavik & Haapasalo 1990, Basrani et al. 2003, Gomes et al. 2003).

It is active against a wide range of microorganisms, such as Gram-positive and Gram-negative bacteria.[30] CHX has an antibacterial efficacy comparable to that of sodium hypochlorite (NaOCl).[65] In addition, it is also effective against strains resistant to $Ca(OH)_2$. CHX may also impart

substantive antibacterial activity to root dentin after prolonged (ie, at least 1 week) exposure.[5, 37, 50, 52, 96] 2% CHX is proved to be an efficient agent against E. faecalis.[44]

Finding healing powers in plants is an ancient idea. Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization.[14] They constitute a promising source of phytotherapy drugs and new molecules.[23] Their beneficial effects allied to the current worldwide "back to nature" trend have led to greater attention being paid to these products.[29] Numerous studies have identified compounds within herbs that are effective antibiotics (Basile et al., 2000; Cowan, 1999). Traditional healing systems around the world that utilize herbal remedies are important resources for the discovery of new antibiotics (Okpekon et al., 2004). Certain traditional remedies have already been reported to be effective against drug-resistant bacteria (Kone et al., 2004; Sato et al., 2000).

Interestingly, the effects of fruit extracts on bacteria have been studied by numerous researchers world-wide (Reddy et al., 2001; Erdoorul, 2002; Atefl and Erdoorul, 2003). In addition, much work has been focused on ethnomedicinal plants in India (Maheshwari et al., 1986; Rai, 1989; Negi et al., 1993). It has been suggested that ethanol and aqueous extracts from

plants are potential sources of antimicrobial agents (Chung et al., 1995; Vlietinck et al., 1995).[86]

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have lead to the screening of several medicinal plants for their potential antimicrobial activity.[77] Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs.[14] The number of studies on this alternative therapeutic system increased in the last decades, as well as their use for several purposes.[23]

Triphala is a traditional Siddha herbal formulation consisting of the dried fruits of three medicinal plants Terminalia chebula, Terminalia bellerica and Phyllanthus emblica, also known as the three myrobalans. Triphala means three (tri) fruits (phala). It is reported to have various biological activities as: Anti-oxidant, Anti-cancer, Anti-mutagenic, Immunomodulatory, Anti-allergic, Radioprotective, Adaptogenic, Cardiotonic, Hypocholesterolaemic, Capillary strengthening, Hepatoprotective, Anti-bacterial, Anti-viral, Anti-fungal, Anti-malarial.[41, 76]

Terminalia chebula is one of the constituents of Triphala. Terminalia chebula is a plant species belonging to the genous Terminalia, family

Combretaceae. It is a flowering evergreen tree called in English the black myrobalan. It is also known as Haritaki (Sanskrit and Bengali), Harad (Hindi), Karkchettu (Telugu), Kadukkai (Tamil), Harada (Marathi & Gujrati). It is native to Indian subcontinent and the adjacent areas such as Pakistan, Nepal and the South-West of China stretching as far south as Kerala or even Sri Lanka where it is called Aralu. The fruit of the tree has been used as traditional medicine for household remedy against various human ailments, since antiquity. Terminalia chebula has been extensively used in Indian medicine and has become a cynosure of modern medicine.[14]

Antibacterial activity of Terminalia chebula against both Gram positive and Gram negative human pathogenic bacteria has also been reported. Aqueous extract of Terminalia chebula exhibits antifungal activity against a number of dermatophytes and yeasts. It is effective against the pathogenic yeast Candida albicans.[94]

This knowledge would enable more rational exploitation of this plant material both in traditional medicine and in the empirical development of new antimicrobials. The present systematic and methodical investigations have therefore been designed to elucidate the possible antibacterial activity of alcoholic extracts of Triphala and Terminalia chebula against E. faecalis and its potential to be used as an intracanal medicament to eliminate

E. faecalis from infected root canals and aid in the ultimate success of endodontic treatment.

AIMS & OBJECTIVES

1. To evaluate the potential of antimicrobial activity of Triphala and T. chebula against E. faecalis, by,

- Assessment of Minimum Inhibitory Concentration (MIC) of Triphala and T. chebula against E. faecalis.
- Assessment of the levels of susceptibility of E. faecalis to Triphala, T. chebula and CHX by measuring the zone of inhibition.

2. To evaluate the potential of Triphala & T. chebula to be used as an intracanal medicament, against E. faecalis biofilms formed on dentin samples, by,

- Assessing the antimicrobial activity at 200 µm and 400 µm depths by Colony Count (CFU/ml) and Optical Density values (OD) measured using spectrophotometer.
- Comparative evaluation of the activity of these herbal extracts with 2% CHX.

REVIEW OF LITERATURE

Enterococcus faecalis & Intracanal Medicaments:

Haapasalo et al. (1987)[35] developed an in vitro model for dentinal tubule infection of root canals. Cylindrical dentin specimens, 4mm high with a diameter of 6 mm and a canal 2.3 mm wide, were prepared from freshly extracted bovine incisors. The cementum was removed from all dentin blocks. The tubules were opened by four-minute treatments with 17% EDTA and 5.2% NaOCl before being infected with Entercoccus faecalis ATCC 29212 in yeast extract-glucose broth. Bacteria rapidly invaded the tubules. After three weeks of incubation, a heavy infection was found 400 µm from the canal lumen and the front of the infection reached 1000 µm in some blocks. Camphorated paramonochlorophenol (CMC) and a calcium hydroxide compound, Calasept, were tested for their disinfecting efficacy toward E. faecallis – infected dentin. Liquid CMCP rapidly and completely disinfected tubules, whereas CMCP in gaseous form disinfected tubules less rapidly. Calasept failed to eliminate, even superficially, E. faecalis in the tubules. The method used in bacteriological sampling allowed for sequential removal of 100 µm thick zones of dentin from the central canal towards the periphery. The control specimens were uniformly infected and yielded

growth in bur samples up to some 500 µm from the surface. The model proved quite sensitive and seems suitable for in vitro testing of root canal medicaments.

Chong et al. (1992)[16] discussed the role of intracanal medication as a root canal dressing. They stated that in pulpectomy and some root canal treatments, where the root canal contains vital pulp tissue, it is doubtful whether a routine intracanal medicament is needed. In infected root canals, intracanal medication has been advocated for many purposes. An intracanal medicament is used to: (i) eliminate any remaining bacteria after canal instrumentation; (ii) reduce inflammation of periapical tissues and pulp remnants; (iii) render canal contents inert and neutralize tissue debris; (iv) act as a barrier against leakage from the temporary filling; (v) help to dry persistently wet canals. The authors concluded that intracanal medicaments should only be used for root canal disinfection as part of controlled asepsis in infected root canals, and their role is secondary to cleaning and shaping of the root canal. Thorough canal debridement and adequate canal preparation are more pertinent and their importance is emphasized. Bacteriological sampling may be necessary if a tooth does not respond to treatment, to help in the choice of intracanal medicament.

Gomes et al. (1996)[32] studied variation in the susceptibilities of endodontic microflora to chemical procedures and confirmed that organisms like E. faecalis were recovered from canals after thorough Bio Mechanical Preparations. He concluded certain organisms like E. faecalis are difficult to be eliminated from infected root canals.

Sundqvist et al. (1998)[88] conducted a study to determine what microbial flora were present in teeth after failed root canal therapy and to establish the outcome of conservative re-treatment. Fifty-four root-filled teeth with persisting periapical lesions were selected for re-treatment. After removal of the root filling, canals were sampled by means of advanced microbiologic techniques. The teeth were then re-treated and followed for up to 5 years. The results showed that the microbial flora was mainly single species of predominantly gram-positive organisms. The isolates most commonly recovered were bacteria of the species Enterococcus faecalis. The overall success rate of re-treatment was 74%. They concluded that the microbial flora in canals after failed endodontic therapy differed markedly from the flora in untreated teeth. Infection at the time of root filling and size of the periapical lesion were factors that had a negative influence on the prognosis. Three of four endodontic failures were successfully managed by re-treatment.

Lima et al. (2001)[49] conducted an in vitro study to evaluate the effectiveness of chlorhexidine- or antibiotics-based medications in eliminating E. faecalis biofilms using Biofilm Susceptibility Assay. One-day and three-day biofilms of E. faecalis were induced on cellulose nitrate membrane filters. Each biofilm-containing membrane was thoroughly covered with 1 ml of the test medications and incubated for 1 day at 37°C. Treated biofilms were then aseptically transferred to vials containing a neutralizing agent in saline solution and vortexed. Suspensions were 10-fold diluted, seeded onto Mitts salivarius agar plates, and the colony-forming units counted after 48 h of incubation. The results of the study showed that there were significant differences between the formulations tested. The association of clindamycin with metronidazole significantly reduced the number of cells in 1-day biofilms. However of all medications tested, only 2% chlorhexidine-containing medications were able to thoroughly eliminate most of both 1-day and 3-day E. faecalis biofilms. The authors concluded that chlorhexidine may exert an important role in the eradication of endodontic infection associated with teeth that were refractory to conventional endodontic therapy.

Love et al. (2001)[55] postulated that virulence factor of E. faecalis in failed endodontically treated teeth may be related to the ability of E. faecalis

cells to maintain the capability to invade dentinal tubules and adhere to collagen in the presence of human serum. E. faecalis virulence may also be related to its resistance to intracanal medicaments, adherence to host cells, expression of protein to ensure cell survival as a result of altered nutrient supply, ability to compete with other bacterial cells, alter host response and environment.

Bettina Basrani et al. (2002)[7] conducted an in vitro study to assess the substantive antimicrobial activity of different medicaments in human root dentin using spectrophotometric analysis of optical density. Canals of 98 roots were enlarged to standard size and medicated for 7 days with the following: (1) 2% chlorhexidine (CHX) gel, (2) 0.2% CHX gel, (3) 2% CHX solution, (4) Ca(OH)2, (5) Ca(OH)2+ 0.2% CHX gel, (6) 2% CHX solution + a 25% CHX-containing controlled-release device, (7) saline and (8) gel vehicle. After medication, canals were inoculated with Enterococcus faecalis for 21 days. Dentin samples were collected with Gates-Glidden burs into brain heart infusion broth and bacterial growth was assessed with spectrophotometric analysis of optical density after 72 hours of incubation. The results of the study showed that mean optical densities were significantly lower for groups with 2% CHX (1,3, and 6) when compared with those of the controls Other groups did not differ significantly from the

controls. The authors concluded that canal dressing for 1 week with 2% CI IX may provide residual antimicrobial activity against E. faecalis.

Cruz et al. (2002)[18] conducted an in vitro study to evaluate the penetration of propylene glycol into root dentine. Safranin O in propylene glycol and in distilled water was introduced into root canals with and without artificial smear layer. Dye diffusion through dentinal tubules was determined spectrophotometrically. The time required for dye to exit through the apical foramen using propylene glycol and distilled water as vehicles was also determined. The extent and areas of dye penetration on the split surfaces of roots were assessed using Adobe Photoshop and NTH Image Software. The results of the study showed that propylene glycol allowed dye to exit faster through the apical foramen. The area and depth of dye penetration with propylene glycol was significantly greater than with distilled water. Smear layer significantly delayed the penetration of dye. The authors concluded that propylene glycol delivered dye through the root canal system rapidly and more effectively indicating its potential use in delivering intracanal medicaments.

Bettina Basrani et al. (2003)[6] conducted an in vitro study to assess the antimicrobial activity of different medicaments in human root dentin. The effect of CHX (0.2% and 2% in gel or solution) and Ca(OH)2 (alone or

with 0.2% CHX gel) was evaluated by using the agar diffusion test and an in vitro human root inoculation method, to measure zone of inhibition or bacterial growth with optical density by spectrophotometric analysis, respectively. For optical density analysis, samples from infected root canals were collected after 7 days of medication and were cultured for 24 hours in brain-heart infusion to detect viable bacteria. In the agar diffusion test, CHX was effective against E faecalis in a concentration-dependent fashion, but Ca(OH)2 alone had no effect. In the root canal inoculation test, CHX was significantly more effective against E faecalis than Ca(OH)2 was ($P < .05$), but there were no significant differences between the modes of medication or concentrations of CHX. The authors concluded that CHX is effective against E faecalis in vitro. Further in vivo studies are needed to confirm the value of CHX in clinical treatment.

Gomes et al. (2003)[34] evaluated the effectiveness of 2% chlorhexidine gluconate gel and calcium hydroxide as intracanal medicaments against E.faecalis. The specimens were divided into four groups, according to the intracanal medicament used, as follows: Group 1: 2% chlorhexidine gluconate gel; Group 2: calcium hydroxide in a viscous vehicle (polyethylene glycol 400); Group 3: 2% chlorhexidine gluconate gel+calcium hydroxide and Group 4: Brain Heart Infusion (BHI) broth

(control group). The medicaments were placed into the canal lumen and left there for experimental times of 1, 2, 7, 15 and 30 days. The results of the study showed that chlorhexidine gel alone completely inhibited the growth of E.faecalis after 1,2,7 and 15 days. Calcium hydroxide allowed microbial growth at all experimental times. The combination of chlorhexidine and calcium hydroxide was effective after 1 and 2 days demonstrating 100% antibacterial action; however, its antibacterial activity reduced between 7 and 15 days. The authors concluded that under the conditions of this study, 2% chlorhexidine gel alone was more effective against E. faecalis than calcium hydroxide. However, its antibacterial activity depended on how long it remained inside the root canal.

Isabelle Portenier et al. (2003)[40] Stated that in the past few years, Enterococcus faecalis has been the focus of increased interest both in medicine and dentistry and a recognized pathogen in post-treatment endodontic infections, E. faecalis is frequently isolated both in mixed flora and in monocultures. E. faecalis is probably the species that can best adapt to and tolerate the ecologically demanding conditions in the filled root canal. Eradication of E. faecalis from the root canal with chemomechanical preparation and using disinfecting irrigants and antibacterial dressings is difficult. They concluded that in endodontics, E. faecalis is rarely present in

primary apical periodontitis, but it is the dominant microorganism in root-filled teeth presenting with post-treatment apical periodontitis. It is often isolated from the root canal in pure culture, but it can also be found together with some other bacteria or yeasts. When in mixed infections, E. faecalis typically is the dominant isolate. While there is no doubt about the pathogenicity of E. faecalis in endodontic infections, it seems to be rarely associated with acute infections and flare-ups. Eradication of E. faecalis from the root canal remains a challenge, while chlorhexidine and combinations of disinfectants show some promise.

Vivacqua-Gomes et al. (2005)[93] conducted a study to assess the presence of Enterococcus faecalis after root canal treatment in single or multiple visits in an ex vivo model. Forty-five premolar teeth were infected ex vivo with E.faecalis for 60 days. The canals were then prepared and irrigated with 2% chlorhexidine gel. The specimens were divided into five groups (Gl, G2, G3, G4 and G5) according to the time elapsed between chemical-mechanical preparation and root canal filling, the irrigant solution used and the use or nonuse of a calcium hydroxide intracanal medicament. The teeth were then root-filled and incubated for 60 days at 37°C. Dentine chips were removed from the canal walls with sequential sterile round burs at low speed. The samples obtained with each bur were immediately

collected in separate test tubes containing Brain-Heart Infusion broth. These samples were placed onto agar plates and colony forming units were counted after 24 hours at 37°C. The results of the study showed that Enterococcus faecalis was recovered from all die five groups, with the lowest count being of Gl (chlorhexidine irrigation and immediate root filling in a single visit). The authors concluded that neither single- nor multiple-visit root canal treatment ex vivo, eliminated E. faecalis completely from dentinal tubules. Up to 60 days after root filling, E. faecalis remained viable inside dentinal tubules. When no sealer was used, E. faecalis presented a higher growth rate.

Kayaoglu et al. (2005)[48] conducted an in vitro study to evaluate the effect of growth at pH levels from 7.1 to 9.5 on the adherence of Enterococcus faecalis to bovine serum albumin (BSA) and collagen type I. Enterococcus faecalis strain A197A was grown in broth of adjusted pHs varying between 7.1 and 9.5. Aliquots of bacterial suspensions were added to wells coated either with BSA or with collagen type I. Bacteria adhering to the surfaces were stained with crystal violet. Spectrophotometric measurements of the dissolved stain were used to assess the number of bacteria adhering to the surfaces. The results of the study showed that the adhesion of E. faecalis to BSA-coated surfaces decreased inversely with alkalinity of the growth medium. The pH 7.1-grown bacteria bound to BSA

significantly more than the other BSA groups. On the contrary, the adhesion to collagen type I-coated surfaces of bacteria grown at pH 8.0 and 8.5 was significantly greater than for those grown at pH 7.1. The authors concluded that a minor increase in pH up to 8.5, which may be a consequence of insufficient treatment with alkaline medicaments such as calcium hydroxide, increases the collagen-binding ability of E. faecalis, in vitro. This can be a critical mechanism by which E. faecalis predominates in persistent endodontic infections.

Edgar Schafer et al. (2005)[19] conducted an in vitro study to investigate the efficacy of chlorhexidine (CHX) and calcium hydroxide (Ca(OH)2) against Enterococcus faecalis using culture plate method by counting the colony forming units. Extracted single-rooted human teeth were instrumented up to size 40. After removal of the smear layer, an inoculum of E. faecalis was inserted into the root canals. After incubation, the inoculum was removed and the root canals were filled with one of three different disinfectants. Ca(OH)2 paste, CHX 2%, and a mixture of CHX and Ca(OH)2 paste (n = 10 in each group). Control teeth were filled with water of standardized hardness (n = 10). The teeth were then incubated for 3 days. After incubation, each root canal was instrumented, and the removed dentin was examined microbiologically. The results of the study showed that CHX

was significantly more effective against E. faecalis than was Ca(OH)2 paste or a mixture of CHX with Ca(OH)2 paste. There was no increase in the efficiency of Ca(OH)2 paste when CHX was added. The authors concluded that the results suggest that CHX is effective in the elimination of E. faecalis from dentinal tubules.

Sedgley et al. (2005)[9] studied survival of E. faecalis in root canals environment. Enterococcus faecalis inoculated into root canals maintained viability for 12-months ex vivo. The clinical implications are that viable E. faecalis entombed at the time of root filling could provide a long-term nidus for subsequent infection. The conclusion of this study was that E. faecalis entombed at the time of root filling could provide a long term nidus for subsequent infection.

Ferrari et al. (2005)[25] attempted to detect enterococci, enteric bacteria and yeast species from the canals of teeth with primary endodontic infections before and after canal preparation. They concluded that Enterococci, enteric bacteria and yeasts were present in primary endodontic infections. Enterococci, particularly Enterococcus faecalis and E. faecium were resistant to removal by root canal preparation followed by intracanal dressing.

George et al. (2005)[28] studied the role of environmental changes on monospecies biofilm formation on root canal wall by E.faecalis. The bioflim mode of growth is a survival strategy and harsh environmental conditions existing in root canal may favor the growth of bacteria as a biofilm. This aspect is supported by the fact that clinically isolated E. faecalis possess increased adhering capacity, increased virulence factor & increased resistance to antimicrobials that are all characteristics of biofilm style of growth. In this study, it was concluded that biofilm forming capacity of microorganisms depends upon the surface attributes of the substratum and can vary according to prevailing environmental and nutritional conditions.

Sathorn et al. (2006)[10] discussed to what extent does calcium hydroxide intracanal medication eliminate bacteria from human root canals, compared with the same canals before medication, as measured by the number of positive cultures, in patients undergoing root canal treatment for apical periodontitis. The results showed that in eight studies included in the review, covering 257 cases; sample size varied from 18 to 60 cases; six studies demonstrated a statistically significant difference between pre-and post-medicated canals, whilst two did not. There was considerable heterogeneity among studies. The difference between pre- and post-medication was not statistically significant. The authors concluded that

Calcium hydroxide has limited effectiveness in eliminating bacteria from human root canal when assessed by culture techniques. The quest for better antibacterial protocols and sampling techniques must continue to ensure that bacteria have been reliably eradicated prior to obturation.

Ertugrul Ercan et al. (2006)[21] conducted an in vitro study to compare the effectiveness of various medicaments, including Ca(OH)2 with 2% chlorhexidine, 2% chlorhexidine gel and Ca(OH)2 alone, against Enterococcus faecalis and Candida albicans by counting the colony forming units . Eighty extracted single-rooted human maxillary teeth were taken and instrumented. The root canal was irrigated with EDTA solution to remove smear layer. Then, roots were infected with E.faecalis and C.albicans. Subsequently, the roots were divided into 4 treatment groups: group 1 was treated with calcium powder hydroxide in distilled water, group 2 was treated with calcium hydroxide powder in 2% chlorhexidine, group 3 was treated with 2% chlorhexidine gel and group 4 was treated with 0.9% sterile saline serving as negative control. Microbial samples were taken after 7, 15 and 30 days. After incubation, dentine chips were obtained from each root canal and examined microbiologically The microbiological samples were plated to count colony-forming units in per milligram of dentin The results of the study showed that the 2% chlorhexidine gel was significantly more

effective than calcium hydroxide with 2% chlorhexidine, calcium hydroxide with distilled water and control saline solution. The authors concluded that under the conditions of this study, 2% chlorhexidine gel is effective in the elimination of E.faecalis calls and C.albicans from the root canal system. However, to support this in vitro observation, further in vivo studies are needed.

Lisane Paquette et al. (2007)[53] conducted an in vivo study to assess the antibacterial efficacy of intracanal medication with 2% chlorhexidine liquid (CHX) in teeth with apical periodontitis. Canals in 22 teeth were instrumented at the first session, medicated with CHX and reaccessed after 7 to 15 days. Bacteriological samples were aspirated at the first and second sessions, before (1A, 2A) and after (IB, 2B) canal instrumentation. Viable bacterial counts were obtained by culture (CFU) and microscopy using vital dyes. The results of the study showed that microscopic counts were higher than CFU counts. Consistently high CFU counts in 1A samples decreased significantly in IB samples, increased significantly in 2A samples and decreased in 2B samples to the level of IB samples. Proportions of negative cultures followed the pattern of CFU counts. Intracanal medication with chlorhexidine did not reduce the bacterial concentration. The authors concluded that 2% chlorhexidine gluconate liquid applied in vivo as

intracanal medication for 7 days to 15 days did not increase the proportion of teeth with negative cultures or reduce the bacterial counts beyond that achieved after chemomechanical preparation in the first treatment session. Although the bacterial regrowth was less than in historic controls, the potential benefits of CHX indicated by in vitro studies were not fully realized. Thus, further research is warranted on different delivery forms of chlorhexidine, as well as alternative intracanal medications.

Wang et al. (2007)[15] conducted a study to evaluate the clinical efficacy of 2% chlorhexidine (CHX) gel on intracanal bacteria reduction during root canal instrumentation using culture plate method by counting the colony forming units. The additional antibacterial effect of an intracanal dressing (Ca[OH]2 mixed with 2% CHX gel) was also assessed. Forty-three patients with apical periodontitis were recruited. Four patients with irreversible pulpitis were included as negative controls. Teeth were instrumented using rotary instruments and 2% chlorhexidine gel as the disinfectant. Bacterial samples were taken upon access (SI), after instrumentation (S2) and after 2 weeks of intracanal dressing (S3). Anaerobic culture was performed. The results of the study showed that four samples showed no bacteria growth at S1, which were excluded from further analysis. Of the samples cultured positively at SI, 10.3% (4/39) and 8.3%

(4/36) sampled bacteria at S2 and S3, respectively. A significant difference in the percentage of positive culture between SI and S2 but not between S2 and S3 was found. The authors concluded that the results suggest that 2% chlorhexidine gel is an effective root canal disinfectant and additional intracanal dressing did not significantly improve the bacteria reduction on the sampled root canals.

Jogikalmat Krithikadatta et al. (2007)[44] conducted an in vitro study to evaluate the disinfection of dentinal tubules using 2% chlorhexidine gel, 2% metronidazole gel, bioactive glass (S53P4) in comparison with calcium hydroxide. The antibacterial efficacy of the four medicaments against Enterococcus faecalis was assessed in Vitro using culture plate method by counting the colony forming units in extracted premolar teeth at the depths of 200 µm and 400 µm. The results of the study showed that the overall percentage inhibition of bacterial growth (at 200 µm and 400 µm depth) was 100% with 2% chlorhexidine gel. The inhibition of growth was moderate with 2% metronidazole gel (86.5%), followed by bioactive glass (62.8%) and calcium hydroxide (58.5%). The authors from the present study concluded that 2% chlorhexidine gel alone was most effective against E. faecalis when compared to other medicaments tested.

Souza-Filho et al. (2008)[26] conducted an in vitro study to evaluate the effectiveness of 2% chlorhexidine (CHX) gluconate gel, calcium hydroxide [Ca(OH)2] and their combination with iodoform and zinc oxide powder as intracanal medications against select microorganisms using agar diffusion method and to measure the pH changes caused by these medications. The zones of growth inhibition were measured. The pH of the pastes was measured right after preparation, after 24 h and 1 week later. The results of the study showed that the largest mean zones of microbial inhibition were produced by 2% CHX gel, followed by Ca(OH)2 + 2% CHX gel + iodoform, Ca(OH)2 + 2% CHX gel, Ca(OH)2 + 2% CHX gel + zinc oxide and Ca(OH)2 + water. The mean pH of all medications stayed above 12.0 during the whole experiment, except for chlorhexidine gel (pH=7.0). All medications had antimicrobial activity, but the most effective against the tested microorganisms were 2% chlorhexidine gel, followed by its combination with Ca(OH)2 and iodoform. The authors concluded that Ca(OH)2 associated with 2% chlorhexidine, with or without iodoform or zinc oxide, when used as an intracanal medication provides antimicrobial action and is able to maintain an ideal pH.

Herbal Extracts:

Ahmad et al. (1998)[3] subjected a total of 82 Indian medicinal plants traditionally used in medicines to preliminary antibacterial screening, against several pathogenic and opportunistic microorganisms. Aqueous, hexane and alcoholic extracts of each plant were tested for their antibacterial activity using agar well diffusion method at sample concentration of 200 mg/ml. The results indicated that out of 82 plants, 56 exhibited antibacterial activity against one or more test pathogens. Interestingly, extracts of five plants showed strong and broad spectrum activity as compared to rest of 51 plant extracts which demonstrated moderate activity. On the whole the alcoholic extracts showed greater activity than their corresponding aqueous and hexane extracts. Among various extracts, only alcoholic extracts of Emblica officinalis, Terminalia chebula, Terminalia bellerica, Plumbago zeylanica and Holarrhena antidysenterica were found to show potentially interesting activity against test bacteria. These active crude alcoholic extracts were also assayed for cellular toxicity to fresh sheep erythrocytes and found to have no cellular toxicity.

Jagtap et al. (1999)[41] conducted a study to test the ability of the aqueous extract of T. chebula to inhibit the growth and some physiological functions of Streptococcus mutans. The extract strongly inhibited the growth, sucrose

induced adherence and glucan induced aggregation of S. mutans. Mouth rinsing with a 10% solution of the extract inhibited the salivary bacterial count and salivary glycolysis. Mouth rinsing with the extract significantly reduced total bacterial counts and the total streptococcal counts in the saliva samples obtained up to and including 3 h after rinsing, compared with the counts obtained pre-rinsing or after placebo rinsing. The extract successfully inhibited glycolysis of salivary bacteria for up to 90 min post-rinsing.

Naik et al. (2004)[63] conducted a study to assess the potential antioxidant activity of aqueous extract of a natural herb, Terminalia chebula by examining its ability to inhibit γ-radiation-induced lipid peroxidation in rat liver microsomes and damage to superoxide dismutase enzyme in rat liver mitochondria. The antimutagenic activity of the extract has been examined by following the inhibition of γ-radiation-induced strand breaks formation in plasmid pBR322 DNA. In order to understand the phytochemicals responsible for this, HPLC analysis of the extract was carried out, which showed the presence of compounds such as ascorbate, gallic acid and ellagic acid. This was also confirmed by cyclic voltammetry. The extract inhibits xanthine/xanthine oxidase activity and is also an excellent scavenger of DPPH radicals. The rate at which the extract and its constituents scavenge the DPPH radical was studied by using stopped-flow kinetic spectrometer.

Based on all these results it is concluded that the aqueous extract of T. chebula acts as a potent antioxidant and since it is able to protect cellular organelles from the radiation-induced damage, it may be considered as a probable radioprotector.

K.M. Elizabeth. (2005)[20] conducted a study to assess the antimicrobial activity of crude and methanol extract of Terminalia bellerica dry fruit by disc diffusion method, against 9 human microbial pathogens. Crude aqueous extract of dry fruit at 4 mg concentration showed zone of inhibition ranging from 15.5-28.0 mm. S. aureus was found to be highly susceptible forming highest zone of inhibition, suggesting that T. bellerica was strongly inhibitory towards this organism. These pathogens were highly sensitive to the methanol extract forming 14.0 to 30.0 mm zone of inhibition suggesting that the methanol extract of T. bellerica was more effective than crude extract against most of the microbes tested except E. coli (enteropathogen) and P. aeruginosa. The minimal inhibitory concentrations (MICs) of crude and methanol extracts were determined by broth dilution technique which ranged from 300 to >2400 μg/ml and 250 μg to >2000 μg/ml respectively, indicating that T. bellerica was highly effective against S. aureus with lower MIC values. There were some biochemical alterations induced by T.

bellerica. These results indicate that T. bellerica dry fruit possesses potential broad spectrum antimicrobial activity.

Hyun-Sun LEE et al. (2005)[38] stated that The ripe fruit of Terminalia chebula RETZIUS (T. chebula RETZ) (Combretsceae), which is a native plant in India and Southeast Asia, has traditionally been used as a popular folk medicine for homeostatic, antitussive, laxative, diuretic, and cardiotonic treatments. They conducted a study to evaluate the protective effects of an aqueous extract of fruit of T. chebula on the tert-butyl hydroperoxide (t-BHP)-induced oxidative injury observed in cultured rat primary hepatocytes and rat liver. Both treatment and pretreatment of the hepatocytes

with the T. chebula extract (TCE) significantly reversed the t-BHP-induced cell cytotoxicity and lactate dehydrogenase leakage. In addition, TCE exhibited in vitro ferric-reducing antioxidant activity and 2,2-diphenyl-1-picryhydrazyl free radical-scavenging activities. The in vivo study showed that pretreatment with TCE (500 or 1000 mg/kg) by gavage for 5 d before a single dose of t-BHP (0.1 mmol/kg i.p.) significantly lowered the serum levels of the hepatic enzyme markers aspartate aminotransferase and alanine aminotransferase and reduced the indicators of oxidative stress in the liver, such as the glutathine disulfide content and lipid peroxidation, in a dose dependent manner. Histopathologic examination of the rat livers showed that

TCE reduced the incidence of liver lesions, including hepatocyte swelling and neutrophilic infiltration, and repaired necrosis induced by t-BHP. Based on the results described above, we speculate that TCE has the potential to play a role in the hepatic prevention of oxidative damage in living systems.

Tasduq et al. (2006)[90] stated that Terminalia chebula (Combetraceae) is an important herbal drug in Ayurvedic pharmacopea. In their study, a 95% ethanolic extract of T. chebula (fruit) (TC extract), which was chemically characterized on the basis of chebuloside II as a marker, was investigated for hepatoprotective activity against anti-tuberculosis (anti-TB) drug-induced toxicity. TC extract was found to prevent the hepatotoxicity caused by the administration of rifampicin (RIF), isoniazid (INH) and pyrazinamide (PZA) (in combination) in a sub-chronic mode (12 weeks). The hepatoprotective effect of TC extract could be attributed to its prominent anti-oxidative and membrane stabilizing activities. The changes in biochemical observations were supported by histological profile.

Srikumar et al. (2007)[87] stated that, the isolation of microbial agents less susceptible to regular antibiotics and the rising trend in the recovery rates of resistant bacteria highlights the need for newer alternative principles. Triphala has been used in traditional medicine practice against certain diseases such as jaundice, fever, cough, eye diseases etc. In their study

phytochemical (phenolic, flavonoid and carotenoid) and antibacterial activities of aqueous and ethanol extracts of Triphala and its individual components (Terminalia chebula, Terminalia bellerica and Emblica officinalis) were tested against certain bacterial isolates (Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella sonnei, S. flexneri, Staphylococcus aureus, Vibrio cholerae, Salmonella paratyphi-B, Escherichia coli, Enterococcus faecalis, Salmonella typhi) obtained from HIV infected patients using Kirby-Bauer's disk diffusion and minimum inhibitory concentration (MIC) methods. T. chebula was found to possess high phytochemical content followed by T. bellerica and E. officinalis in both aqueous and ethanol extracts. Further, most of the bacterial isolates were inhibited by the ethanol and aqueous extracts of T. chebula followed by T. bellerica and E. officinalis by both disk diffusion and MIC methods. It revealed that both individual and combined aqueous and ethanol extracts of Triphala have antibacterial activity against the bacterial isolates tested.

Chattopadhyay et al. (2007)[14] stated that Terminalia chebula is a great herb with lack of extensive research studies. Despite this fact it has been in use as the most frequently used herb in Ayurveda. They studied the antibacterial activity of aqueous, hexane and ethanol extracts of black myrobalan (fruit of Terminalia chebula Retz.) by agar-well diffusion method against

uropathogen Escherichia coli of clinical origin. It was observed that both aqueous and ethanolic extracts showed strong (IZD = 21+1.41mm – 24.0 mm) and hexane extract mild (IZD = 9+1.41 mm) zone of inhibition against the strain evaluated. The minimal inhibitory concentration (MIC) values in macrobroth dilution assay technique of aqueous and ethanolic extracts were 6.25 mg/ml and 3.12 mg/ml respectively. Minimal bactericidal concentration (MBC) values were two to four fold higher than the corresponding MIC values and the total activity of the aqueous and ethanolic extracts were 67.5 ml and 154.7 ml respectively. Thus, both aqueous and ethanolic extracts of black myrobalan were strongly inhibitory towards the uropathogen E. coli with bactericidal activity (MBC ranging from 6.25 mg/ml to 25.0 mg/ml) against the test strain and ethanolic extract was found to possesses highest antibacterial activity in comparison with its aqueous counterpart on the basis of their MIC index and total activity values and these findings reinforce the importance of ethnomedical approach as a potential source of bioactive substances against urinary tract infections caused by first major bacterial culprit Escherichia coli.

Rasool et al. (2007)[75] Attempted to evaluate the antiarthritic effect of the Indian Ayurvedic herbal formulation Triphala on adjuvant-induced arthritis in mice and to compare it with that of the non-steroidal antiinflammatory

drug indomethacin. Triphala (1 g/kg/bxwt) and indomethacin (3 mg/kg/bxwt) were administered orally for 8 days (from day 11 to 18) after adjuvant injection. The levels of lysosomal enzymes, tissue marker enzymes, glycoproteins and paw thickness were increased in adjuvant-induced arthritic animals. The body weight was found to be reduced when compared with the control animals. These physical and biochemical changes observed in arthritic animals were altered significantly to near normal conditions after oral administration of Triphala (1 g/kg/b.wt). The results obtained clearly indicate the fact that the Indian Ayurvedic herbal formulation Triphala has promising anti-inflammatory activity.

Usha et al. (2007)[12] stated that Plant-derived medicines have been a part of our traditional health care system, and the antimicrobial properties of plant-derived compounds are well documented. They conducted a study to evaluate the effect of an aqueous extract of Terminalia chebula (a medicinal plant) on salivary samples and its potential for use as an anticaries agent in the form of mouthwash. A concentrated aqueous extract was prepared from the fruit of T. chebula. A mouth rinse of 10% concentration was prepared by diluting the extract in sterile distilled water. The efficacy of the mouth rinse was assessed by testing on 50 salivary samples. Salivary samples were collected from subjects assessed to be at high risk for caries. Salivary pH,

buffering capacity, and microbial activity were assessed before rinsing, immediately after, and 10 min. 30 min. and 1 h after rinsing. There was an increase in the pH and buffering capacity and decrease in microbial count. An aqueous extract of T. chebula used as a mouth rinse seems to be an effective anticaries agent.

Biradar et al. (2008)[97] conducted a study to evaluate Antimicrobial activity of aqueous and alcoholic extracts of both Triphala and Triphala Mashi. Comparative phytochemical profile of Triphala and Triphala Mashi was done by preliminary phytochemical screening, total phenolic content and thin layer chromatography (TLC). Antimicrobial activity includes isolation of pathogens from clinical samples, its characterization, testing its multiple drug resistance against standard antibiotics and antimicrobial activity of aqueous and alcoholic extracts of both Triphala and Triphala Mashi against these organisms by using agar gel diffusion method. They concluded that Triphala Mashi containing phenolic compounds, tannins exhibited comparable antimicrobial activity in relation to Triphala against all the microorganisms tested. It inhibits the dose-dependent growth of Gram-positive and Gram-negative bacteria.

Kumar et al. (2008)[51] Infection is a major problem in the management of wounds. Even though the development of synthetic antimicrobial agents

persists, drug resistance and toxicity hinder their way. Many plants with multi-potent pharmaceutical activities may offer better treatment options, and Triphala (dried fruits of Terminalia chebula, Terminalia bellirica, and Phyllanthus emblica) are potential formulations evaluated for healing activity on infected wound as it possesses numerous activities. Alcoholic extract of Triphala has shown in vitro antimicrobial activity against wound pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, and Streptococcus pyogenes. In their study an ointment was prepared from the Triphala extract (10% w/w) and assessed for in vivo wound healing on infected rat model by rate of healing, bacterial count, biochemical analysis, and expression of matrix metalloproteinases. The treated group showed significantly improved wound closure. Assessment of granulation tissue on every fourth day showed significant reduction in bacterial count with significant level of collagen, hexosamine, uronic acid, and superoxide dismutase in the treated group ($P < 0.01$). Reduction of matrix metalloproteinase expression observed in the treated group by gelatin zymography and immunoblotting confirmed the in vivo assessment. They concluded that the results showed the antibacterial, wound healing, and antioxidant activities of Triphala ointment, necessary for the management of

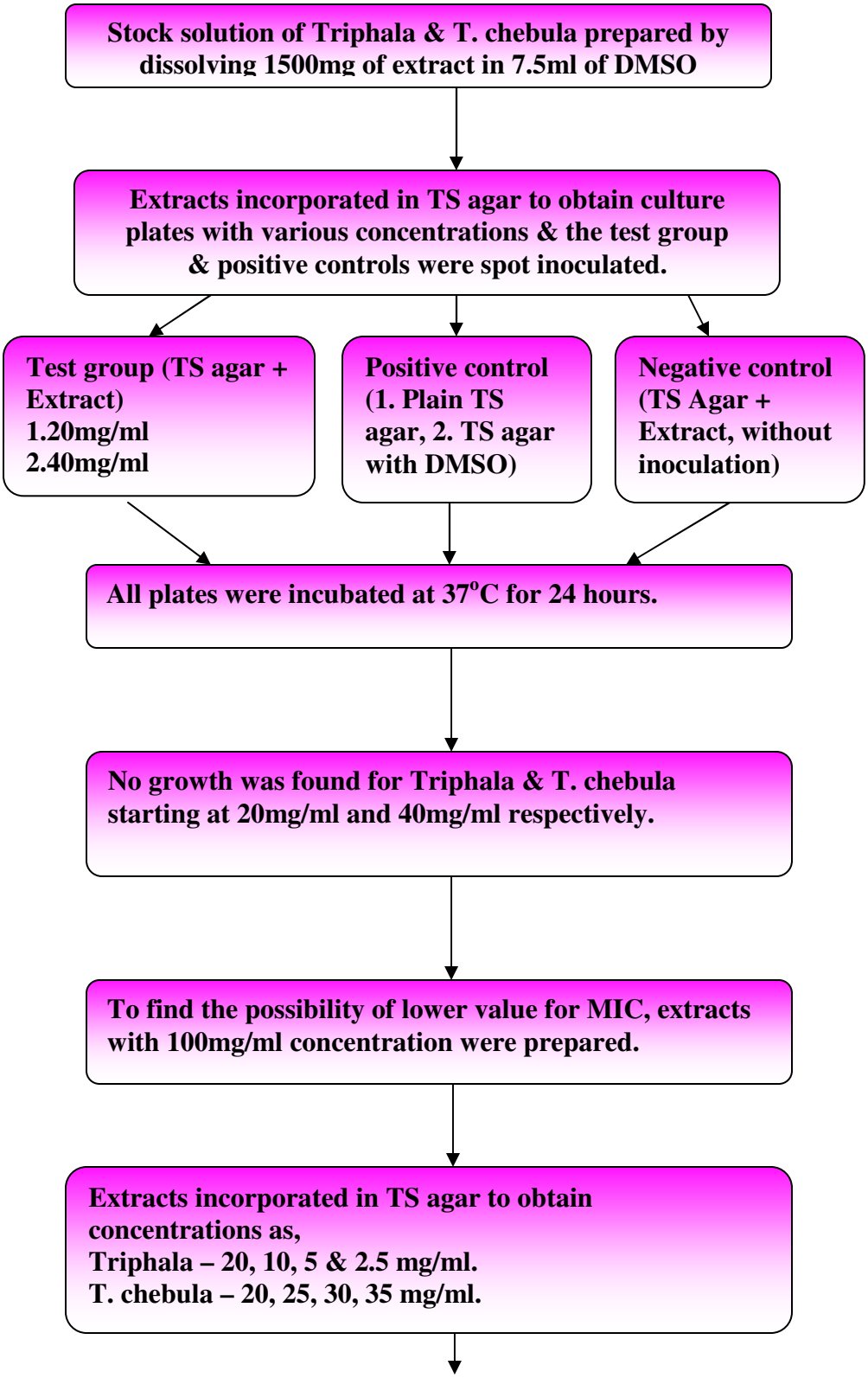
infected wounds. Active principles of the Triphala may be further evaluated and used as an excellent therapeutic formulation for infected wounds.

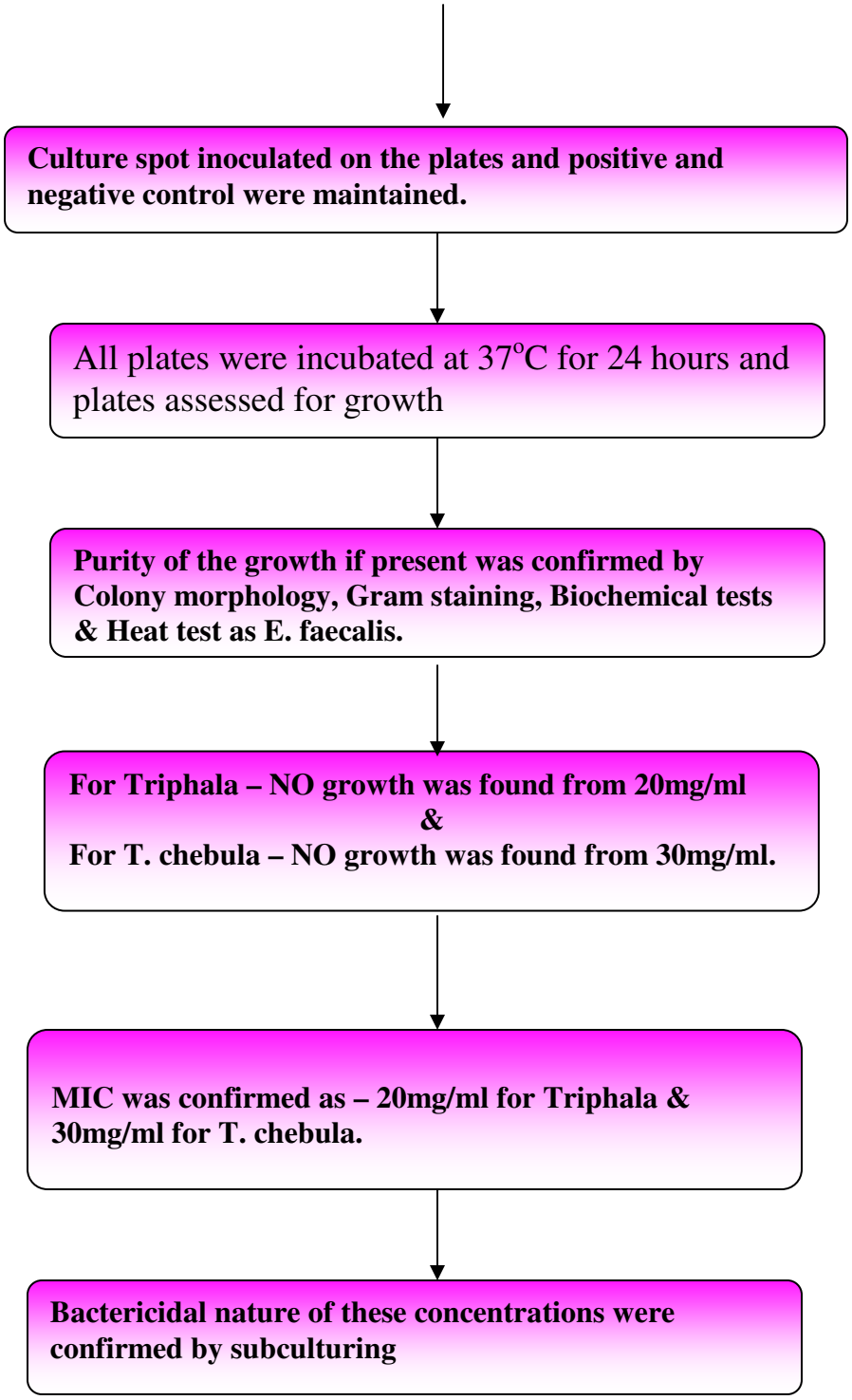
ARMAMENTARIUM

- Culture Plates
- Tripticase Soy broth & agar (Hi Media)
- E. faecalis (ATCC 29212)
- Alcoholic extract of Triphala & T. chebula (Elles Aromatics)
- Chlorhexidine gluconate (Dentachlor)
- Amoxicillin
- Dimethyl Sulfoxide - DMSO (Fischer)
- 28 freshly extracted single rooted human teeth
- Straight hand piece (NSK)
- Contra-angled handpiece (NSK)
- Diamond disk
- K-files (Mani)
- Gates gliddin – ISO size 1, 2, 3, 4 & 5 (Mani)
- 5% NaOCl (prime dent)
- 17% EDTA (pulp dent)
- Saline (0.9% w/v)
- Ultrasonic bath
- Autoclave

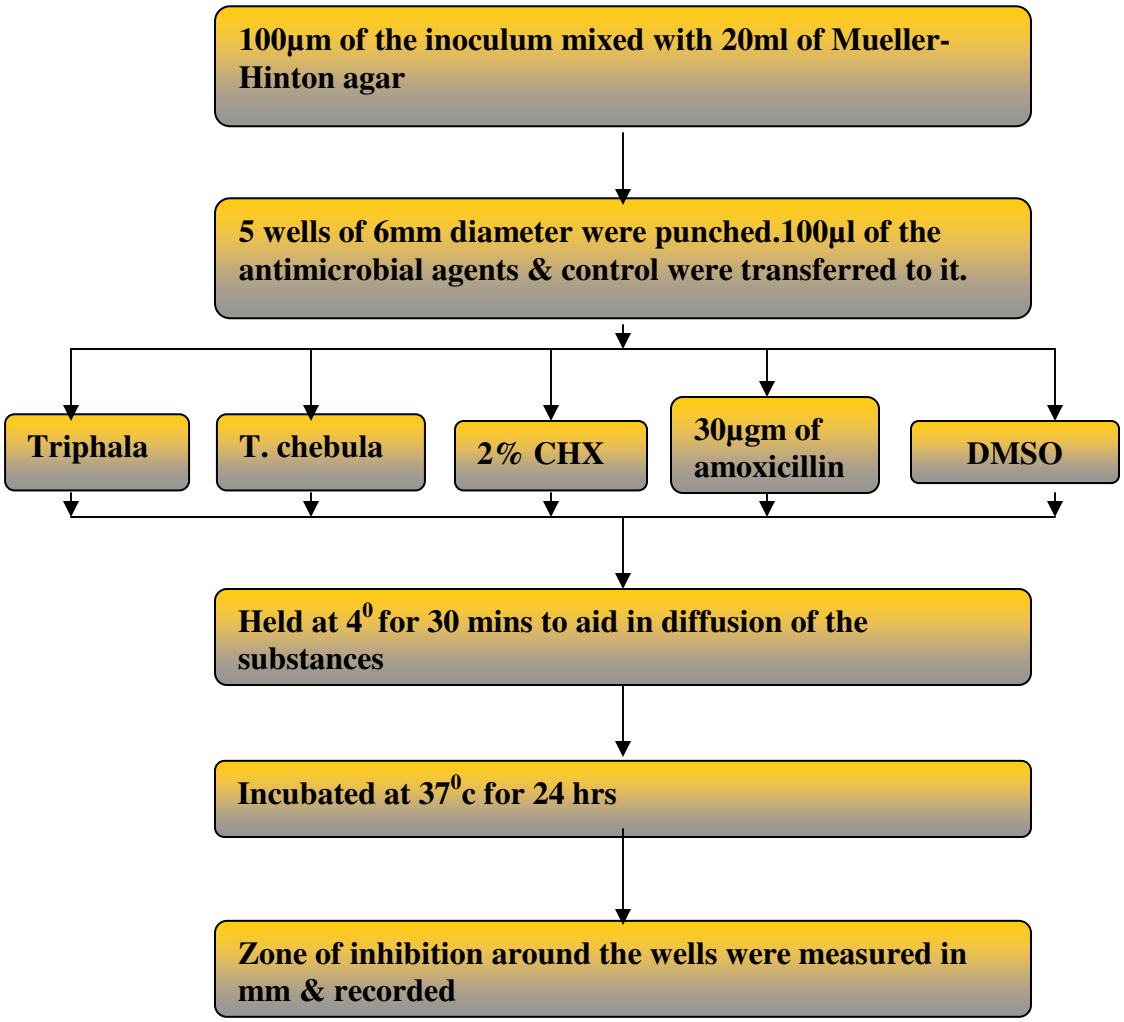
- Incubator (Dalal)
- Laminar Flow
- Micropipette
- Microcentrifuge tube (Tarson)
- Propylene glycol (Fischer)
- Methyl cellulose
- Glass slab & Spatula
- Hand plugger
- Sticky Wax
- Digital colony counter (Dalal)
- Spectrophotometer (Bio-Rad)
- Sterile gloves
- Mask

ASSESSMENT OF MIC

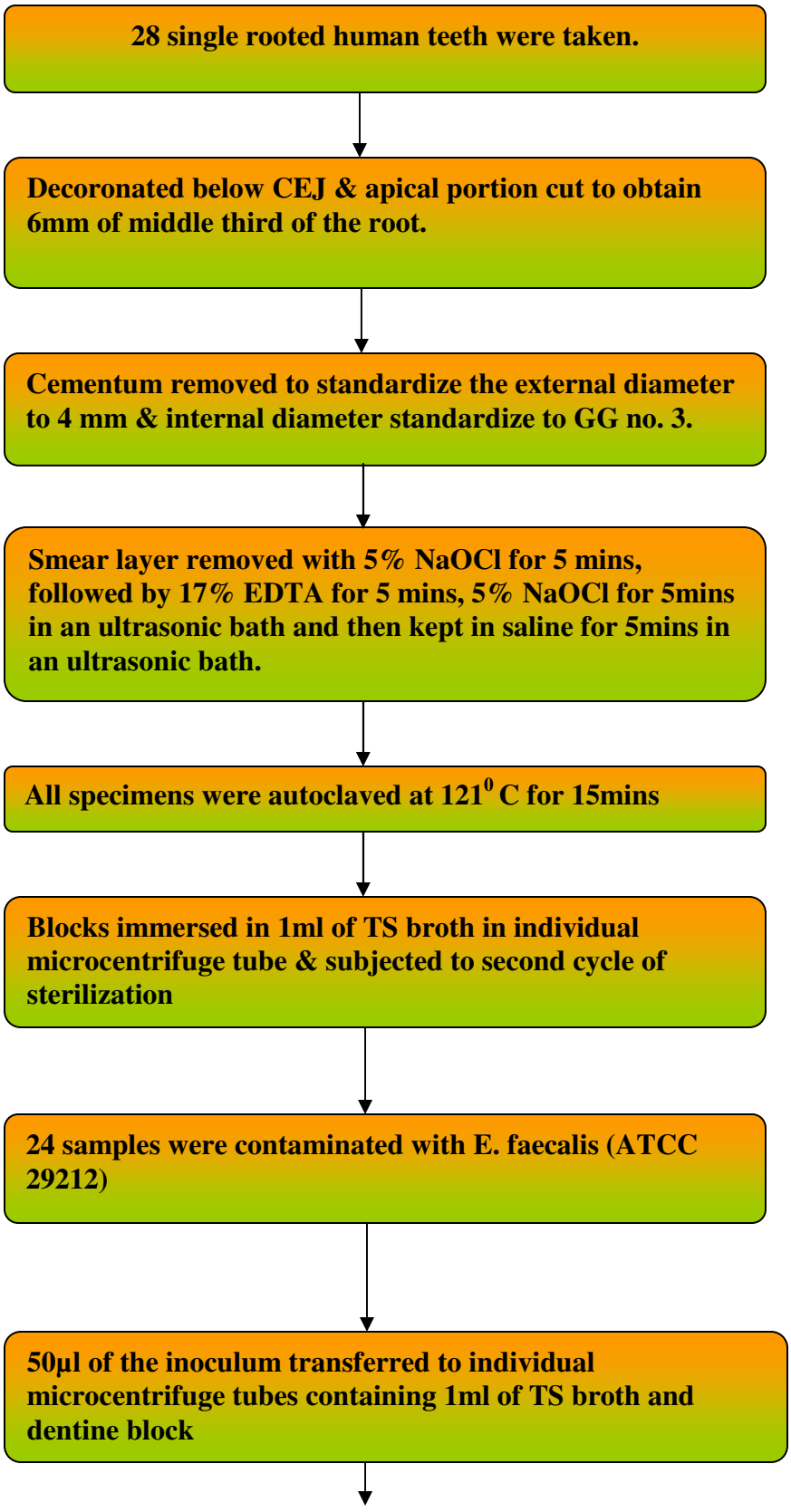


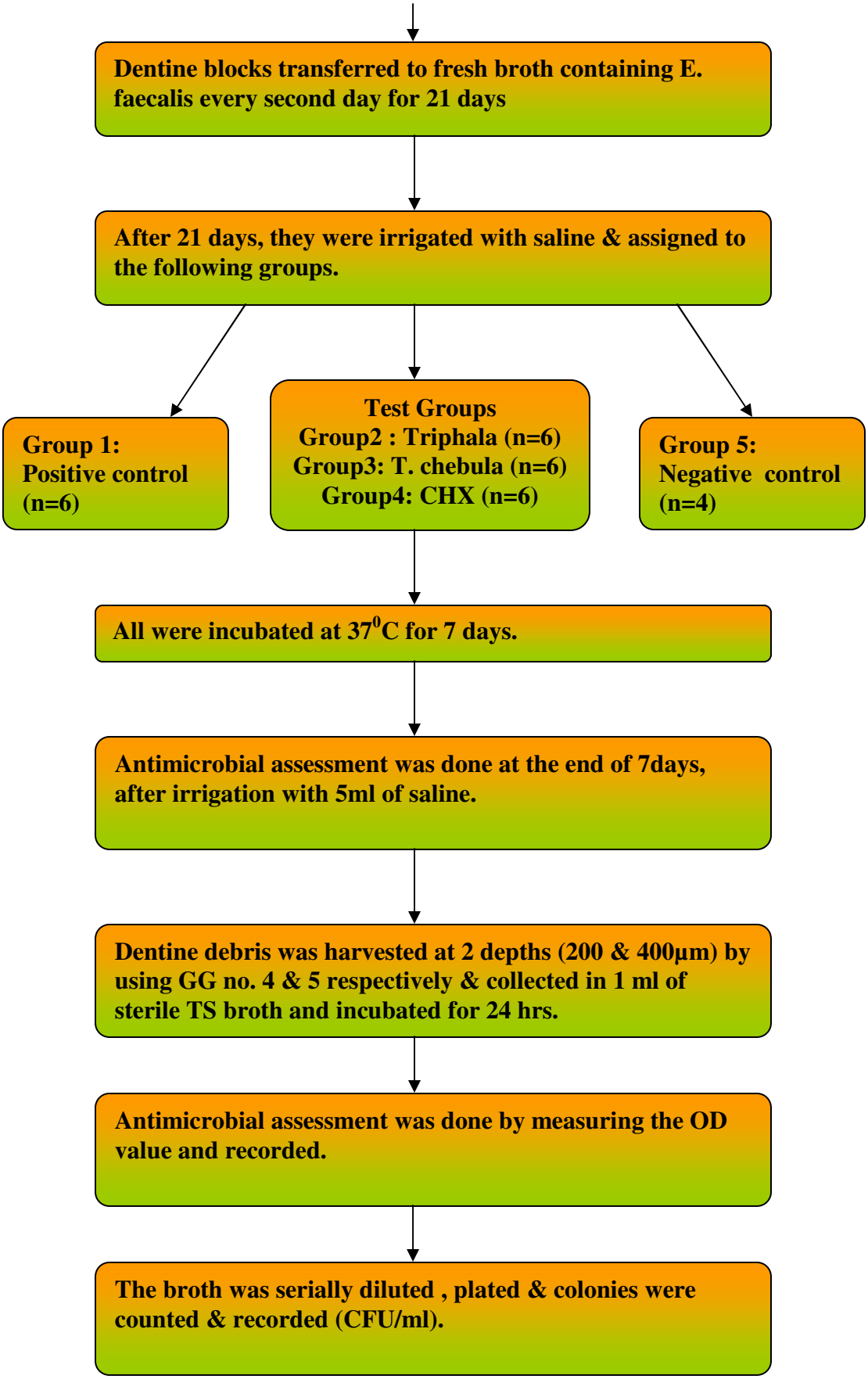

Culture spot inoculated on the plates and positive and negative control were maintained.
All plates were incubated at 37°C for 24 hours and plates assessed for growth
Purity of the growth if present was confirmed by Colony morphology, Gram staining, Biochemical tests & Heat test as E. faecalis.
For Triphala – NO growth was found from 20mg/ml
&
For T. chebula – NO growth was found from 30mg/ml.
MIC was confirmed as – 20mg/ml for Triphala & 30mg/ml for T. chebula.
Bactericidal nature of these concentrations were confirmed by subculturing

SUSCEPTIBILITY TEST BY WELL DIFFUSION



ASSESSMENT OF ANTIMICROBIAL ACTIVITY AGAINST E.FAECALIS BIOFILM IN TEETH SAMPLES




Dentine blocks transferred to fresh broth containing E. faecalis every second day for 21 days
After 21 days, they were irrigated with saline & assigned to the following groups.
Group 1: Positive control (n=6)
Test Groups
Group2 : Triphala (n=6)
Group3: T. chebula (n=6)
Group4: CHX (n=6)
Group 5: Negative control (n=4)
All were incubated at 37^{0}C for 7 days.
Antimicrobial assessment was done at the end of 7days, after irrigation with 5ml of saline.
Dentine debris was harvested at 2 depths (200 & 400µm) by using GG no. 4 & 5 respectively & collected in 1 ml of sterile TS broth and incubated for 24 hrs.
Antimicrobial assessment was done by measuring the OD value and recorded.
The broth was serially diluted , plated & colonies were counted & recorded (CFU/ml).

METHODOLOGY

E. faecalis (ATCC 29212) maintained in stock culture in Institute of Microbiology, Madras Medical College was used in this study. 24 hr growth of E. faecalis grown on Trypticase Soy (TS) agar slope was suspended in 5ml of TS broth and incubated for 4 hours at 37^0C. The culture suspension was adjusted to match the turbidity equivalent to 0.5 McFarland Standard.[44] This was used as the standardized inoculum for all the procedures.

HERBAL EXTRACTS:

Alcoholic extract of Triphala and T. chebula was procured (Elles Aromatics, India). To find the activity of the extract against E. faecalis, the extracts were dissolved in Dimethyl Sulfoxide (DMSO), which is an inert organic solvent and does not have any antibacterial action on its own.[14]

Initially to find the Minimum Inhibitory Concentration (MIC), serial ten-fold dilutions were made to obtain a concentration range from 0.01 to 1000 µg / ml for each extract in TS broth. Then 0.02 ml of the 0.5 McFarland culture suspension was added to them. Two control tubes were maintained. These included a positive control (the tube containing the growth medium, physiological saline and the inoculum) and a negative control (tube containing extract and the growth medium without inoculum). The tubes were incubated at 37^0C for 24 hours. Then 0.1 ml of culture

medium from each tube was subcultured on fresh TS agar plates and incubated for 24 hours. All the plates had growth of microorganisms. This revealed that the concentration tried was insufficient to exhibit antimicrobial activity. Then several attempts were made with various concentration ranges (upto 15mg/ml), to find the MIC.

Finally, a stock solution was prepared by dissolving 1500mg of extract in 7.5ml of DMSO. The extracts were incorporated in the TS agar and culture plates were prepared with concentration ranging from 20mg/ml to 80mg/ml of the extracts by proportioning as in the table.

Extract		Agar		Net concentration of Extract present
0.5 ml	+	4.5 ml	=	20 mg/ml
1 ml	+	4 ml	=	40 mg/ml
1.5 ml	+	3.5 ml	=	60 mg/ml
2 ml	+	3 ml	=	80 mg/ml

Then the culture was spot inoculated on the plates. Positive control was maintained by inoculating a plain TS agar plate without any antibacterial agent. Negative controls were obtained by maintaining an extract incorporated plate and DMSO control (TS agar plate with DMSO) without inoculation of culture over it. The plates were incubated at 37^0C for 24 hours.

The plates were assessed for presence or absence of microbial growth. When growth was present, the organism was confirmed as E. faecalis by colony morphology, gram straining, heat test and standard biochemical tests.[17]

Colony Morphology – It formed small (0.5 to 1 mm) clear colonies on TS agar.

Gram Staining – It was seen as gram positive cocci, occurring in ovoid pairs or in short chains.

Biochemical Tests – Biochemical reactions of it was assessed and it showed :

Arabinose	-
Mannitol	+
Raffinose	-
Sorbitol	+
Urease	+
NH3 produced from arginine	+
Bile Esculin Test	+

Heat Test – It showed growth, even after heating the culture suspension at 60^0C for 30 mins.

All of these features confirmed that the growth that was present in some of the plates was pure culture of E. faecalis.

To confirm the bactericidal nature of that particular concentration, samples were taken from the spotted region with a platinum loop and subcultured on a TS agar plate and incubated for 24 hrs at 37^0C and observed for the presence or absence of growth.

The concentration in which growth was present or absent was noted. The readings were as follows:

Concentration (mg/ml) of extract	Triphala	T. chebula
20	NG	G
40	NG	NG
60	NG	NG
80	NG	NG

At that point of the experiment, no growth was seen from 20 mg/ml of extract concentration for Triphala and 40 mg/ml extract concentration for T. chebula. To explore the possibility of activity at even a slightly lower concentration, the extract with concentration of 100 mg/ml was used to prepare plates with concentrations ranging as in the table.

Triphala

Extract		Agar		Net concentration of extract
1 ml	+	4 ml	=	20 mg
0.5 ml	+	4.5 ml	=	10 mg
0.25 ml	+	4.75 ml	=	5 mg
0.125 ml	+	4.875 ml	=	2.5 mg

T. chebula

Extract		Agar		Net concentration of extract
1 ml	+	4 ml	=	20 mg
1.25 ml	+	3.75 ml	=	25 mg
1.5 ml	+	3.5 ml	=	30 mg
1.75 ml	+	3.25 ml	=	35 mg

The culture plates were spot inoculated, incubated, assessed for growth of organism and subcultured for confirmation of bactericidal activity and the growth when present was assessed for purity of the culture, as done previously.

MIC was taken as the lowest concentration in which no growth was detected. The readings were as follows.

Triphala		*T. chebula*	
20 mg / ml	NG	20 mg / ml	G
10 mg / ml	G	25 mg / ml	G
5 mg / ml	G	30 mg / ml	NG
2.5 mg / ml	G	35 mg / ml	NG

So, the MIC of Triphala was found to be 20 mg / ml and for T. chebula 30 mg / ml.

SUSCEPTIBILITY TEST BY WELL DIFFUSION METHOD

100 µl of inoculum adjusted to 0.5 McFarland was mixed in 20 ml of Mueller-Hinton agar and shaken. 0.5µg/ml of methylene blue was added to it, to impart colour to the medium, which may aid in the ease of assessment of the readings. Then the media was poured in a sterilized petridish . 5 wells of 6 mm diameter were punched into the agar medium with sterile metal cylinder. 100 µl of the extracts dissolved in DMSO, adjusted to the concentration of MIC was transferred to the wells. 100 µl of 2 % chlorhexidine and 30 µg of amoxicillin in the wells served as positive control and DMSO served as negative control. After holding the plates at 4^0C for 30 mins to aid in diffusion of the antimicrobial agents into the

medium,[2] they were incubated at 37^0C for 24 hours and then the diameter of zone of microbial growth inhibition around the wells were measured in millimeters and recorded.

ASSESSMENT OF ANTIMICROBIAL ACTIVITY AGAINST E. FAECALIS BIOFILM IN THE TEETH SPECIMENS:

The dentin block model used in the experiment was modified from the one developed by Haapasalo and Orstavik (1987). In the present study, extracted human teeth were used instead of bovine incisors (Safavi et al, 1990; Weiger et al, 2002; Lui et al, 2004; Saleh et al, 2004)[89]. Freshly extracted single rooted human teeth stored in 10 % formalin were used.

Preparation of The Dentin Blocks:

A rotary diamond disk was used to decoronate the teeth below the cemento-enamel junction. The remaining root was then sectioned such that 6 mm of the middle third of the root was obtained. Cementum was removed from the root surface for standardizing the external diameter to 4 mm. The internal diameter was standardized to Gates Glidden 3 (Mani Inc, Tachigi – Ken, Japan) in a slow speed handpiece (NSK, Tokyo, Japan).[44] Organic and inorganic debris was removed by treating the blocks in an ultrasonic bath with 5% sodium hypochlorite for 5 mins, followed by 17% Ethylenediamine

tetra-acetic acid for 5 mins and again with 5% sodium hypochlorite for 5 mins. The blocks were immersed in the ultrasonic bath of saline for 5 mins to remove all the traces of the chemicals used and sterilized in an autoclave at 121^0C for 15 mins. The blocks were subjected to a second cycle of sterilization with the blocks immersed in 1 ml of Trypticase Soy (TS) broth in individual microcentrifuge tubes. This allows better penetration of the broth into the dentinal tubules.[44]

Infecting the Dentin Blocks:

50 µl of the 0.5 McFarland inoculum of E. faecalis was transferred to presterilized individual microcentrifuge tubes contating 1 ml of the TS broth and dentin block. The dentin blocks were transferred to the fresh broth containing E. faecalis every second day. All the procedures were carried out under laminar flow. The purity of the culture was checked by subculturing 5 µl of the broth from the incubated dentin block in TS broth on TS agar plates. The dentin blocks were infected for a period of 21 days.[44]

Antimicrobial Assessment:

After the incubation period, the blocks were irrigated with 5 ml of sterile saline to remove the incubation broth. Then the dentin blocks were assigned to the following groups, each containing 6 blocks for the first 4 groups and 4 blocks for group 5.

Group 1 - Positive control (Infected dentin blocks with saline treatment).

Group 2 - Triphala

Group 3 - T. chebula

Group 4 - Chlorhexidine (CHX)

Group 5 - Negative control (Sterile dentin blocks with saline treatment).

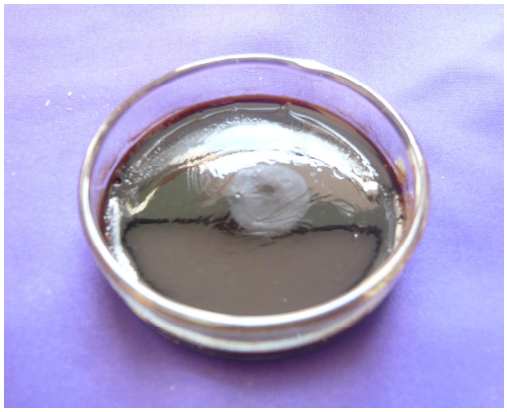
According to Fava and Saunders (1999), the antibacterial activity of intracanal medicament is enhanced by the vehicle used.[24] So, propylene glycol (Fischer) was used as a solvent. 3 gm of extracts were dissolved in 3 ml of propylene glycol, to be used as an intracanal medicament in group 2 and 3. 2% CHX was used in group 4. Methyl cellulose was used as thickening agent,[44] to obtain a paste like consistency, for the extracts and CHX. This was carried into the canal by using a sterile file and condensed with a hand plugger. All of the blocks after medication were sealed above and below with sticky wax and put in individual microcentrifuge tubes (all performed inside a laminar flow chamber) and incubated at 37^0C. The medicament was left in place for 7 days and antimicrobial assessment was performed after it. Then the blocks were washed with 5 ml of sterile saline.

Dentin debris was harvested at 2 depths (200 µm and 400 µm) by using Gates-Glidden drills number 4 and 5 (Mani, Inc) respectively and collected in 1 ml of sterile TS broth in individual microcentrifuge tubes and incubated at 37^0C for 24 hours. After the incubation period, to measure optical density of the broth, 300 µl of it was transferred from each tube into the microtiter plate and optical density measured using spectrophotometer (Bio-Rad) at 620 nm. The OD readings were adjusted to the OD value of fresh and sterile broth as a blank.

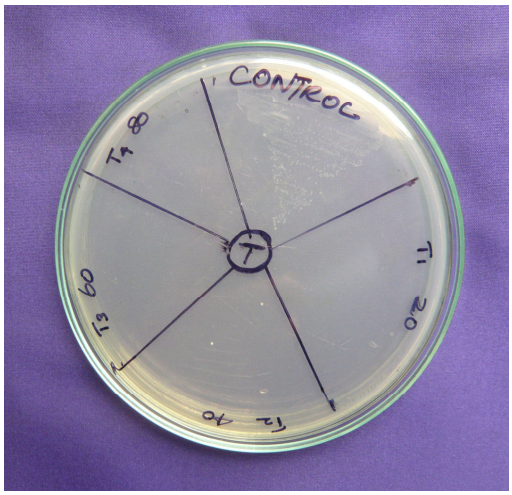
In order to assess the number of colony forming units, the broth was serially diluted, plated on TS agar plates and incubated at 37^0C for 24 hours and the CFU were counted and recorded (CFU/ml).

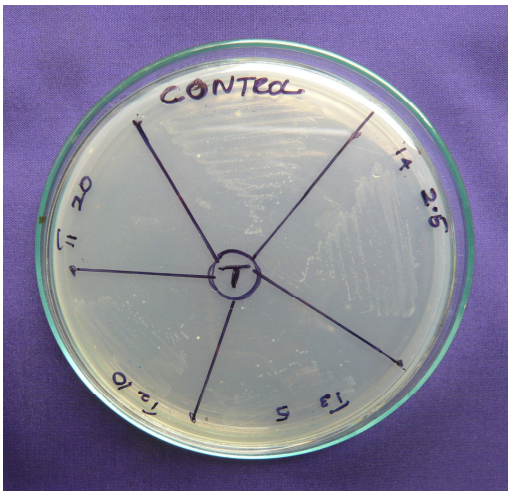
Medicaments used in the study

TS Agar plate with extract

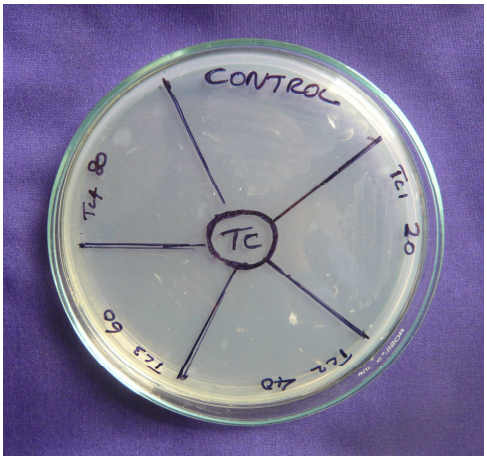


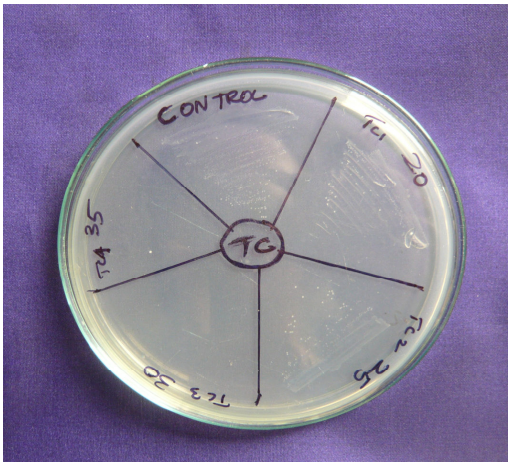
Subculture confirming MIC of Triphala



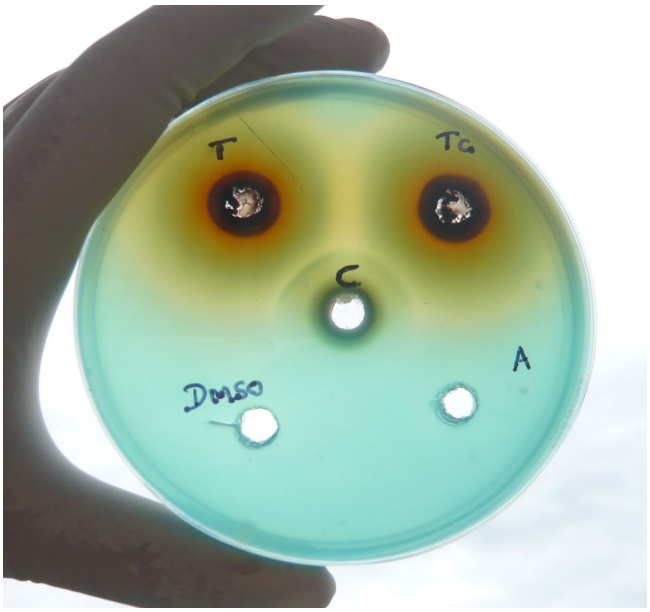


Subculture confirming MIC of T.chebula



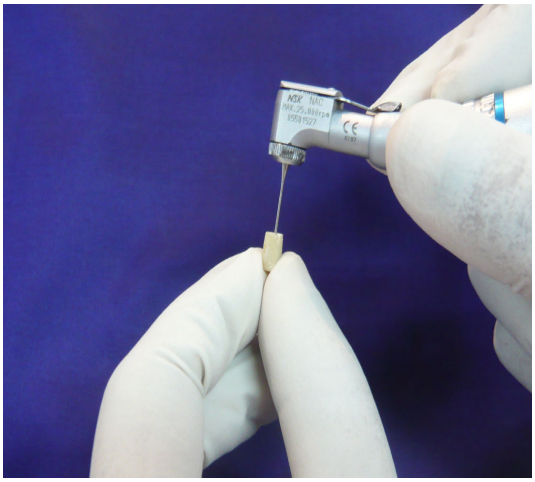


Zone of inhibition

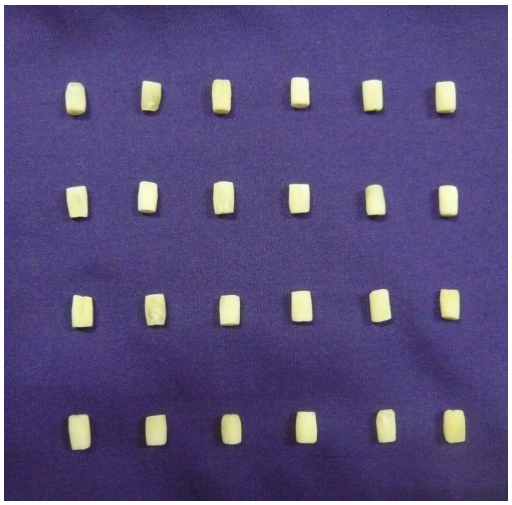


Decoronation & Preparation of dentin block

Internal diameter standardized with GG

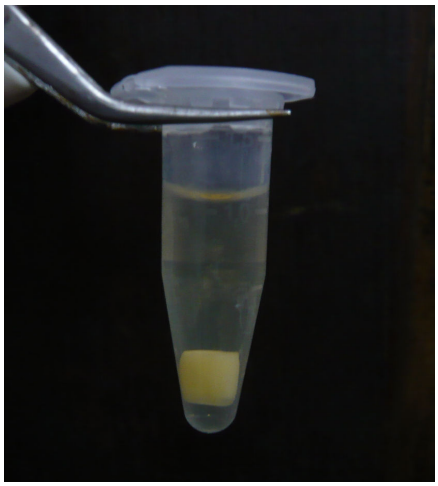


Dentin blocks



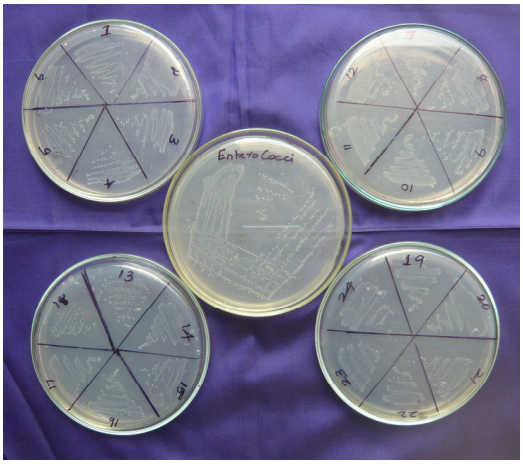
Armamentarium for dentin block preparation

Dentin block in Microcentrifuge tube

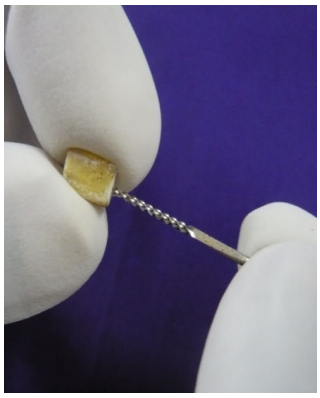


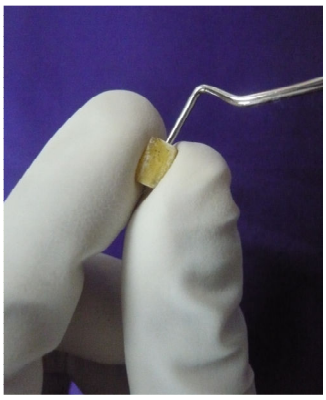
Infected dentin blocks in Incubator

Subculturing to test purity of culture



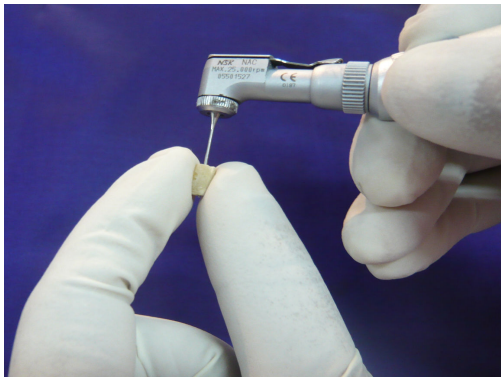
Dentin block Medicated



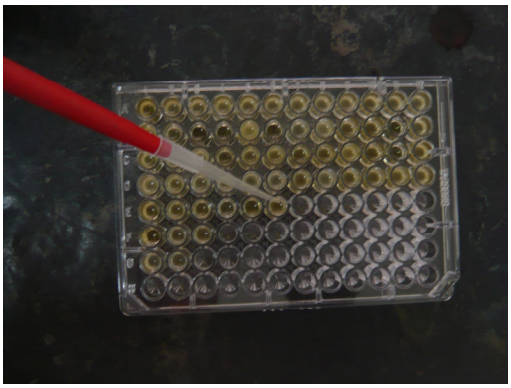


Medicated dentin blocks in Microcentrifuge tubes

Harvesting dentin debris with GG



Broth transferred to microtitre plate

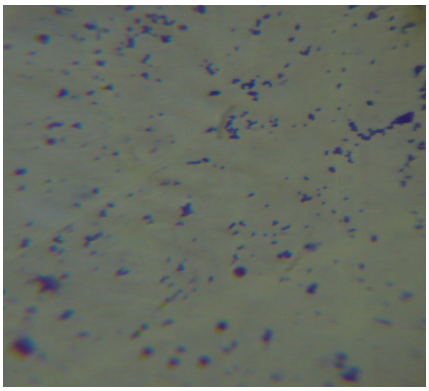


Spectrophotometer

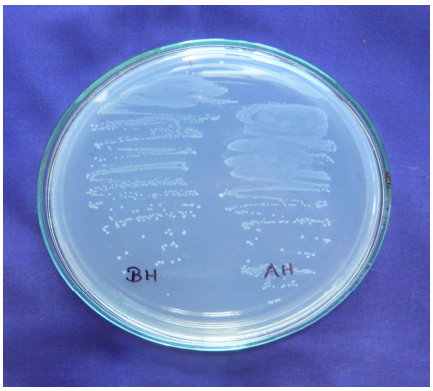
Culture plates for Colony count

Digital Colony Counter

Gram staining



Heat Test



Biochemical Tests

RESULTS & STATISTICAL ANALYSIS

I. The **values of zone of inhibition** produced are presented in Table 1.1.

Table 1.1 - Zone of Inhibition (mm)

Triphala	45	46	45
T. chebula	48	49	47
CHX	25	23	27
Amoxicillin	20	18	21
DMSO	0	0	0

The data were analysed by Oneway Anova followed by Tukey – HSD (Post-Hoc) multiple comparison test and Kruskal-Wallis test.

Table 1.2 - ANOVA

	Mean	SD
Triphala	45.33	0.577
T. chebula	48	1
CHX	25	2
Amoxicillin	19.67	1.528
DMSO	0	0

Table 1.3 - TUKEY – HSD MULTIPLE COMPARISONS

Comparisons	Mean Difference	p value
Triphala vs T. chebula	-2.667	> 0.05
Triphala vs CHX	20.333	< 0.001
Triphala vs AMOX	25.667	< 0.001
Triphala vs DMSO	45.333	< 0.001
T. chebula vs CHX	23.000	< 0.001
T. chebula vs AMOX	28.333	< 0.001
T. chebula vs DMSO	48.000	< 0.001
CHX vs AMOX	5.333	< 0.01
CHX vs DMSO	25.000	< 0.001
AMOX vs DMSO	19.667	< 0.001

Table 1.4 - KURSKAL – WALLIS TEST

	Mean Rank	p value
Triphala	11	< 0.01
T. chebula	14	
CHX	8	
Amoxicillin	5	
DMSO	2	

INFERENCE

- All the antimicrobial agents showed high statistically significant difference ($p < 0.001$) when compared with DMSO.
- There was no statistically significant difference between Triphala and T. chebula ($p > 0.05$)

- Triphala and T. chebula showed high significant difference ($p < 0.001$) when compared with Amoxicillin and CHX.
- CHX showed significant difference ($p < 0.01$) when compared with Amoxicillin.

II. The **OD Values** measured for all the groups at **200 µm & 400 µm** are presented in the Table 2.1 & 2.2.

Table 2.1 – OD Values (200 µm)

Group 1	0.474	0.482	0.486	0.461	0.419	0.456
Group 2	0.003	0	0.001	0.002	0	0
Group 3	0	0.002	0.002	0.001	0	0.001
Group 4	0	0.001	0.002	0.001	0.001	0.003
Group 5	0.001	0	0.002	0.001	-	-

Table 2.2 – OD Values (400 µm)

Group 1	0.468	0.462	0.473	0.458	0.42	0.459
Group 2	0	0.002	0	0.001	0.003	0
Group 3	0.002	0	0.001	0	0.001	0.001
Group 4	0.001	0.028	0.011	0.026	0.042	0.018
Group 5	0	0.001	0	0.002	-	-

The data were statistically analysed using Oneway Anova followed by Tukey – HSD (Post-Hoc) multiple comparison test and Kruskal-Wallis test was done for non-parametric data.

Table 2.3 – ANOVA (OD Values)

Groups	200 µm		400 µm	
	Mean	SD	Mean	SD
Group 1	0.46300	0.024495	0.45667	0.018843
Group 2	0.00100	0.001265	0.00100	0.001265
Group 3	0.00100	0.000894	0.00083	0.000753
Group 4	0.00133	0.001033	0.02100	0.014311
Group 5	0.00100	0.000816	0.00075	0.000957

Table 2.4 - TUKEY – HSD MULTIPLE COMPARISONS

Goups Comparison	200 µm		400 µm	
	Mean Difference	p value	Mean Difference	p value
G1 vs G2	0.462000	< 0.001	0.455667	< 0.001
G1 vs G3	0.462000	< 0.001	0.455833	< 0.001
G1 vs G4	0.461667	< 0.001	0.435667	< 0.001
G1 vs G5	0.462000	< 0.001	0.455917	< 0.001
G2 vs G3	0.000000	> 0.05	0.000167	> 0.05
G2 vs G4	-0.000333	> 0.05	-0.020000	< 0.05
G2 vs G5	0.000000	> 0.05	0.000250	> 0.05
G3 vs G4	-0.000333	> 0.05	-0.020167	< 0.05
G3 vs G5	0.000000	> 0.05	0.000083	> 0.05
G4 vs G5	0.000333	> 0.05	0.020250	> 0.05

Table 2.5 – KRUSKAL – WALLIS TEST (OD Values)

Goups	200 µm		400 µm	
	Mean rank	p value	Mean rank	p value
Group 1	25.50	< 0.01	25.50	< 0.001
Group 2	10.50		9.08	
Group 3	11.17		9.08	
Group 4	13.00		18.42	
Group 5	11.25		8.38	

INFERENCE

At 200µm:

- Group 1 showed highly significant difference ($p < 0.001$) when compared with all other groups.
- Group 2, 3, 4 & 5 did not show any significant difference ($p > 0.05$) among them at 200µm.

At 400µm:

- Group 1 showed highly significant difference ($p < 0.001$) when compared with all other groups.
- Group 2 & 3 did not show any statistically significant difference ($p > 0.05$) between them and when compared with Group 5.

- Group 2 & 3 had statistically significant difference ($p < 0.05$) when compared with Group 4.

III. The **CFU / ml values** measured for all the groups at **200 µm & 400 µm** are presented in the Table 3.1 & 3.2.

Table 3.1 – CFU / ml Values (200 µm)

Group 1 (x 10^9)	156	168	170	148	142	154
Group 2	0	0	0	0	0	0
Group 3	0	0	0	0	0	0
Group 4	0	0	0	0	0	0
Group 5	0	0	0	0	-	-

Table 3.2 – CFU / ml Values (400 µm)

Group 1 (x 10^9)	148	152	164	152	150	160
Group 2	0	0	0	0	0	0
Group 3	0	0	0	0	0	0
Group 4	0	10	0	10	20	0
Group 5	0	0	0	0	-	-

Table 3.3 - ANOVA (CFU / ml)

Groups	200 µm		400 µm	
	Mean	SD	Mean	SD
Group 1 (x 10^9)	156.33	10.985	154.33	6.250
Group 2	0.00	0.000	0.00	0.000
Group 3	0.00	0.000	0.00	0.000
Group 4	0.00	0.000	6.67	8.165
Group 5	0.00	0.000	0.00	0.000

Table 3.4 - TUKEY – HSD MULTIPLE COMPARISONS

Groups Comparison	200 µm		400 µm	
	Mean Difference	p value	Mean Difference	p value
G1 vs G2	156.333 x 10^9	< 0.001	154.333 x 10^9	< 0.001
G1 vs G3	156.333 x 10^9	< 0.001	154.333 x 10^9	< 0.001
G1 vs G4	156.333 x 10^9	< 0.001	147.667 x 10^9	< 0.001
G1 vs G5	156.333 x 10^9	< 0.001	154.333 x 10^9	< 0.001
G2 vs G3	0.000	> 0.05	0.000	> 0.05
G2 vs G4	0.000	> 0.05	-6.667	> 0.05
G2 vs G5	0.000	> 0.05	0.000	> 0.05
G3 vs G4	0.000	> 0.05	-6.667	> 0.05
G3 vs G5	0.000	> 0.05	0.000	> 0.05
G4 vs G5	0.000	> 0.05	6.667	> 0.05

Table 3.5 – KRUSKAL – WALLIS TEST (CFU / ml Values)

Groups	200 µm		400 µm	
	Mean rank	p value	Mean rank	p value
Group 1	25.50	< 0.001	25.50	< 0.001
Group 2	11.50		10.00	
Group 3	11.50		10.00	
Group 4	11.50		15.50	
Group 5	11.50		10.00	

INFERENCE

At 200 µm & 400 µm:

- Group 1 showed highly significant difference ($p < 0.001$) when compared with all the other groups.
- Group 2, 3, 4 & 5 didn't have any statistical significance ($p > 0.05$) among them.

IV. **OD values and CFU / ml** at **200 µm & 400 µm** for each individual group were compared using independent sample test (Student t-test).

Table 4.1 – Independent Samples Test (Student t-Test)

Groups		CFU / ml			OD		
		Mean	SD	p value	Mean	SD	p value
Group 1	200µm	156.33×10^9	10.98484×10^9		0.46300×10^9	0.0245×10^9	
	400µm	154.33×10^9	6.25033×10^9	> 0.05	0.45670×10^9	0.0188×10^9	> 0.05
Group 2	200µm	0	0		0.00100	0.0013	
	400µm	0	0	> 0.05	0.00100	0.0013	> 0.05
Group 3	200µm	0	0		0.00100	0.0009	
	400µm	0	0	> 0.05	0.00083	0.0008	> 0.05
Group 4	200µm	0	0		0.00133	0.0013	
	400µm	6.6667	8.16497	> 0.05	0.02100	0.0143	< 0.05
Group 5	200µm	0	0		0.00100	0.0008	
	400µm	0	0	> 0.05	0.00075	0.001	> 0.05

Table 4.2 – Mann-Whitney Test

	p value	
Group 4	CFU	OD
	200 & 400	200 & 400
	> 0.05	< 0.05

INFERENCE

- There was no statistically significant difference ($p > 0.05$) between 200µm & 400µm values, when assessed by OD or CFU / ml in Group 1, 2, 3 & 5.
- In Group 4, there was no statistical significance ($p > 0.05$) between the 200 µm & 400 µm values, when assessed by CFU / ml. But, there was statistically significant difference ($p < 0.05$) when OD values were analysed.

INFERENCE OF THE STUDY

I Zone of Inhibition:

- All the antimicrobial agents showed high statistically significant difference ($p < 0.001$) when compared with DMSO.
- There was no statistically significant difference between Triphala and T. chebula ($p > 0.05$)
- Triphala and T. chebula showed high significant difference ($p < 0.001$) when compared with Amoxicillin and CHX.
- CHX showed significant difference ($p < 0.01$) when compared with Amoxicillin.

II OD Values:

- Group 1 showed highly significant difference ($p < 0.001$) when compared with all other groups at 200 & 400 µm.
- Group 2, 3, 4 & 5 did not show any significant difference ($p > 0.05$) among them at 200µm.
- Group 2 & 3 did not show any statistically significant difference ($p > 0.05$) between them and when compared with Group 5.
- Group 2 & 3 had statistically significant difference ($p < 0.05$) when compared with Group 4.

- In groups 1, 2, 3 & 5 there was no statistically significant difference ($p > 0.05$) between 200 & 400 µm values, within a group, when assessed by OD values.
- There was statistically significant difference ($p < 0.05$) between 200 & 400 µm values in Group 4.

III CFU/ml Values:

- Group 1 showed highly significant difference ($p < 0.001$) when compared with all the other groups.
- Group 2, 3, 4 & 5 didn't have any statistical significance ($p > 0.05$) among them.
- In all the groups there was no statistically significant difference between 200 µm & 400 µm values, within a group, when assessed by CFU/ml.

DISCUSSION

Microorganisms play a fundamental role in the etiology of pulp and periapical diseases. Their control and elimination are important during endodontic treatment.[47] E. faecalis is probably the species that can best adapt to and tolerate the ecologically demanding conditions in the filled root canals. It is resistant to various antimicrobials. It is considered as a 'star' in post-treatment disease.[40] Because of this the development of effective therapeutic modalities against E. faecalis is desirable. Hence E. faecalis was chosen for the inoculum in this study. In addition, E. faecalis is relatively easy to culture and it has been used successfully in most studies with the original model.[5, 37, 50, 52, 66]

Various modalities are attempted to eliminate residual bacteria from the root canal, which includes high end technologies as with LASERS, PAD and ANILAD. Even though these show promising results, its applicability to all clinical settings and private practice may be difficult because of the exorbitant cost involved. A cost effective way to deal with the same situation is using intracanal medicaments. The use of an intracanal medicament helps in the elimination of bacteria that remains even after cleaning and shaping, there by providing an environment conducive for periapical tissue repair.[16]

Many medicaments were used as intracanal dressings in the past decades and according to their chemical basis, generally fall into the following categories: phenolic derivatives (eugenol, camphorated para-monochlorophenol, camphorated phenol, metacresyl acetate, beechwood creosote), aldehydes (formocresol), halides (iodine–potassium iodide), calcium hydroxide, antibiotics, and various combinations. The most popular intracanal medicament in use currently is calcium hydroxide. However, various studies confirmed the resistance of E. faecalis to Calcium hydroxide. Various newer medicaments are also tried nowadays, as Bioactive glass and Octanidine. 2% CHX has been found to be highly active against E. faecalis.[21, 44] CHX seems to act by adsorbing on to the cell wall of microorganisms and causing the leakage of intracellular components. It also has the property of substantivity.[5, 37, 50, 52, 96]

There has been a worldwide move towards the use of traditional medicines due to concerns over the more invasive, expensive and potentially toxic mainstream practices (WHO, 2002). Numerous studies have identified compounds within herbals that are effective antibiotics (Basile *et al.*, 2000; Cowan, 1999). Traditional healing systems around the world that utilize herbal remedies are an important resource for the discovery of new antibiotics (Okpekon *et al.*, 2004). Certain traditional remedies have already been reported to be effective against drug-resistant bacteria (Kone *et al.*, 2004; Sato *et al.*, 2000). These facts provoked us to

test an herbal preparation as an intracanal medicament, to find the activity against E. faecalis.

Triphala (a Siddha Herbal Formulation) and T. chebula were selected because of their known broad spectrum of antimicrobial activity. Triphala has been used extensively as a drug against a number of diseases (Awasthi and Nath, 1986; Reddy et al., 1990). Triphala is prescribed for various symptoms of infections, fatigue, assimilation and infectious diseases such as tuberculosis, pneumonia, AIDS (El-Mekkawey and Merelhy, 1995), periodontal diseases (Abraham et al., 2005) and has been reported to reduce considerably the damage due to oxidative stresses in experimental rats (Srikumar et al., 2006). Because of its tremendous potential, it is called the 'Wonder Drug'.

T. chebula is reported to have antioxidant and free radical scavenging activities. It is effective against cancer cells[80] and various microorganisms.[56, 87] It is also useful as an anti-caries agent[87], in dermal wound healing[41], improving gastrointestinal motility, anaphylactic shock[84] and in diabetes mellitus.[79] So, it is called the 'King of Medicines'.[14]

Alcoholic extracts of Triphala and T. chebula were selected as they showed high antibacterial activity compared with aqueous extracts, which might possibly be due to the lesser solubility of the active components in

aqueous solutions.[87] DMSO was used as a solvent, as it does not have any antibacterial action on its own,[56] which had been proved in previous studies and in this study by using it as a control. It is a highly polar organic reagent that has exceptional solvent properties for organic and inorganic chemicals. Thus, it helps in complete dissolution of the extracts.

Assessment of MIC was done to find the concentration of the extracts needed to show antibacterial activity. In this study, MIC was found to be 20mg / ml for Triphala and 30mg / ml for T. chebula. But in a previous study, the MIC for Triphala and T. chebula against E. faecalis were found to be as low as 0.1 µg / ml.[87] This could be because of the effects of geographic variations on the phytochemical concentratation.[42]

The agar diffusion method has been widely used to test the antimicrobial activity of dental materials and medicaments.[31] So, this was used to compare the zone of inhibition produced by the antimicrobial agents used in this study. Among them, Triphala & T. chebula showed highly significant difference and proved to be superior when compared with CHX & Amoxicillin. CHX showed superior antimicrobial action when compared with Amoxicillin. There was no statistically significant difference between Triphala & T. chebula. This revealed that, the extracts have superior antimicrobial activity against E. faecalis. The advantage of this method is that, it allows direct comparison of the materials against

the organisms, indicating which material has the potential to eliminate bacteria in the local microenvironment of the root canal system. However, the limitation of this method is that the result not only depends on the toxicity of the material for the particular organism, but also is influenced by the ability of the material to diffuse across the medium.[74] Further, the materials which show antimicrobial activity in culture plates may not have the same when used as an intracanal medicament, because of the intricacies of the canal system and inherent ability of the dentin to have a negative impact on medication performance.[59]

Generally, bacteria are present in a state of biofilm in the root canals. Estimate of 1000 to 1500 times greater resistance for biofilm grown cells than planktonically grown cells have been suggested. This may turn the same medicament which showed activity against planktonic bacteria to be inactive in clinical scenario. The nature of the substrate may also influence the nature of the biofilm. So, we opted to test the activity of the herbal extracts against the biofilm produced in teeth samples which may also help in assessing the activity in different dentin penetration depths.

The experimental model used in this study was adapted from that established by Ørstavik and Haapasalo for infection and disinfection of dentinal tubules. The model was adapted for extracted human teeth rather than bovine incisors. This modification was considered appropriate

because of the marked difference in diameter between the canals of bovine and human teeth and thus in the volume of medicament that can be placed in these canals. Propylene glycol was used as a vehicle for the herbal extracts, as it can deliver the medicaments rapidly and more effectively in the root canal system and within dentinal tubules.[18]

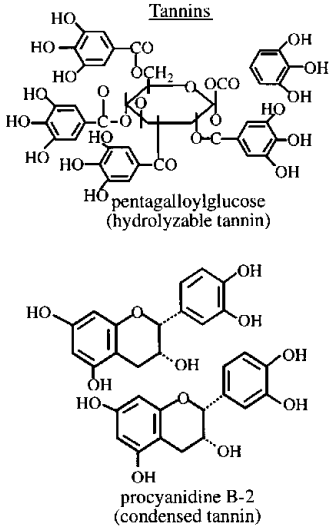
In this study all the medicaments tested showed significant antimicrobial action on E. faecalis when compared with the control group, when assessed by the colony count and OD values. When CFU/ml were considered, Triphala and T. chebula showed 100% inhibition at both 200 µm and 400 µm in all the samples. 2% CHX showed 100% inhibition in all the samples at 200 µm depth and 50% of the samples showed 100% inhibition at 400 µm. There was statistical significance between the test groups and the positive control group. But, there was no statistically significant difference between CHX, Triphala and T. chebula.

When OD values were considered, all the groups showed significant difference with the positive control group. There was no significant difference between CHX, Triphala and T. chebula at 200 µm depth. But, there was statistically significant difference between the herbal extracts and CHX at 400 µm depth. Even though there is no statistically significant difference between test groups when CFU/ml were considered, complete inhibition of the organism at both the depths in all

the Triphala and T. chebula treated specimens may have some clinical significance.

Tannic acid represents the major constituent of the ripe fruit of T. chebula and is present in a concentration of 20–40% (Chopra and Handa, 1958). The chief constituents of tannin are chebulic acid, chebulagic acid, corilagin and gallic acid. Tannin of Terminalia chebula are of pyrogallol (hydrolyzable) type. A group of researchers found 14 components of hydrolyzable tannins (gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulegic acid, chebulinic acid, 1,2,3,4,6- penta-O-galloyl-H-D-glucose, 1,6,-di-O-galloyl-D-glucose, casuarinin, 3,4,6-tri-O-galloyl-D- glucose, terchebulin) from Terminalia chebula fruits.[14] The fruits also contain a variety of carbohydrates, glucose and sorbitol being the major ones. The pericarp of the fruit contains anthraquinone glycosides, saponins, anthrones and anthranols.

Model structure of Tannins



The dried fruits of T. bellerica contain about 20% tannins of both condensed and hydrolysable type. Other constituents identified in the fruit include lipids, b-sitosterol, saponins, gallic and ellagic acids and their derivatives, glycosides and various carbohydrates.

Phyllanthus emblica also contains hydrolysable tannins (emblicanin A, emblicanin b, punigluconin 12% and pedunculagin).

The total tannin content of the extracts used in this study was found to be 42.25% w/w for Triphala and 42.80% w/w for T. chebula. The antibacterial activities of tannins are well documented. Tannins inhibited the growth of many fungi, yeasts, bacteria and viruses. Tannins in these fruits thus serve as natural defense mechanisms against microbial infections.[14]

One of their molecular actions is to complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, their mode of antimicrobial action, may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc.[56] Because of this the antibacterial activity could be attributed to either inhibiting the cell division or to damaging the cell walls of bacteria.[87] They also complex

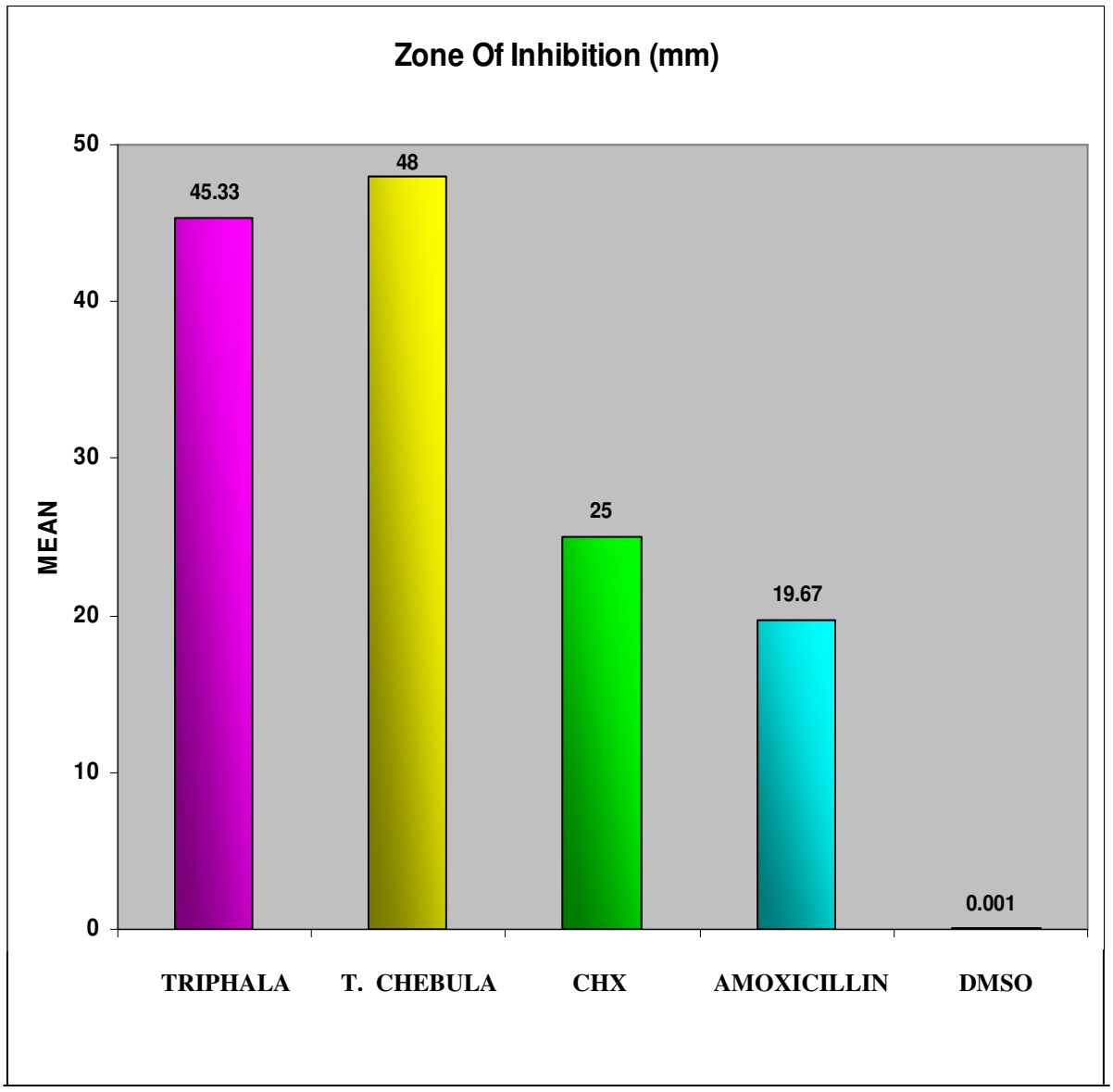
with polysaccharide. The antimicrobial significance of this particular activity was not explored earlier.[56] We speculate that this property of tannins may aid in complexing with glycocalyx (Extracellular polymeric substance which contains polysaccharides and surrounds the microcolonies and anchor the bacterial cell to the substrate) and disrupt biofilm, which may greatly aid in enhancing the antimicrobial action over biofilm. From the literature it has been noted that T. chebula exhibited significant hepatoprotective, cardioprotective, antimutagenic/anticarcinogenic,[80] cytoprotective, radioprotective, antioxidant[63] and adaptogenic[76, 84] effects indicating that it is a safe substance to be used as a drug.[14]

Literature review regarding safety of these herbal extracts stated that Crude alcoholic extracts of T. chebula, T. bellerica and P. Emblica were found to lack cellular toxicity in an assay using fresh sheep erythrocytes.[3] T. chebuala does not exert any cytotoxic effect in allium model. T. chebula by itself had no genotoxic effect both in VITOTOX test and Ames assay.[14] The aqueous extract of T. bellerica fruit was found to be non-toxic when administered orally to mice, whereas the LD50 of the alcoholic extract was equivalent to 4.25g crude drug per kilo body weight. A water soluble fraction of T. bellerica showed no signs of toxicity in mice at oral doses up to 3.2g/kg bodyweight. These facts

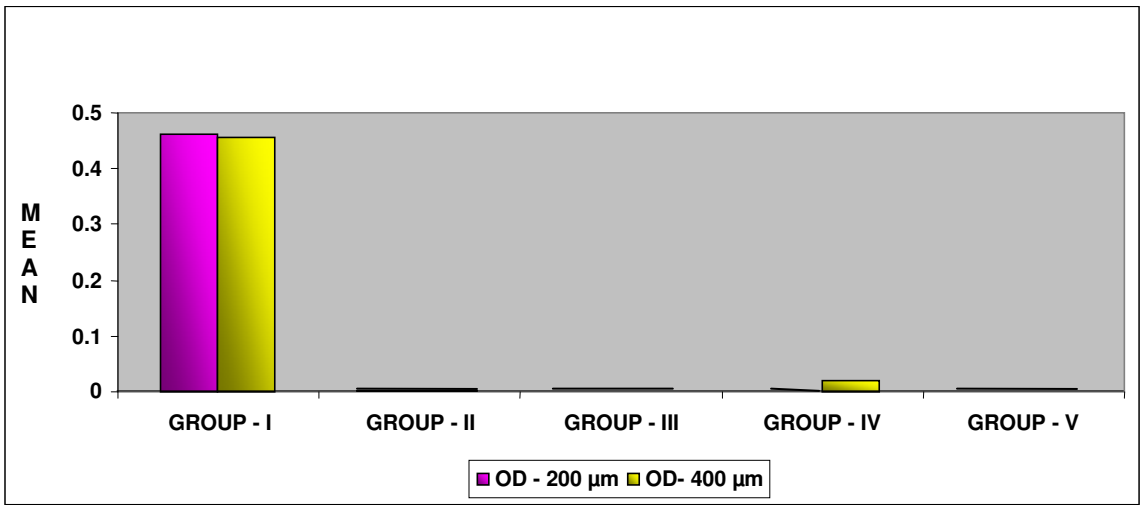
reveal that, it can be safely used as intracanal medicament, even in invivo settings.

These extracts also have antimicrobial action on various other bacteria (gram positive and gram negative), fungi and viruses,[20, 87] which may help in canal disinfection, even in polymicrobial infection, invivo. These extracts also have anti-inflammatory property[75] and wound healing potential.[51] So, they may have a positive influence on minimizing post-operative pain and favour periapical healing, which can be areas of interest for future research.

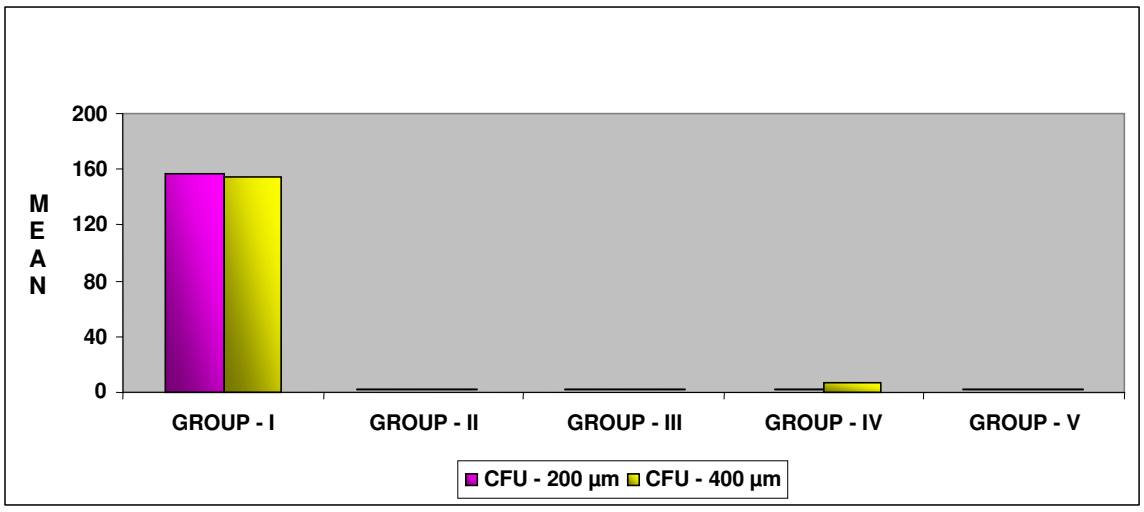
Further exploration should be done on these herbs, which can enable us to utilize the gift of divine nature.


Zone Of Inhibition (mm)
MEAN
50
40
30
20
10
0
45.33
48
25
19.67
0.001
TRIPHALA
T. CHEBULA
CHX
AMOXICILLIN
DMSO

Comparison of OD values between Groups at 200 µm & 400 µm



Comparison of CFU/ml between Groups at 200 µm & 400 µm



RESULTS & STATISTICAL ANALYSIS

I. The **values of zone of inhibition** produced are presented in Table 1.1.

Table 1.1 - Zone of Inhibition (mm)

Triphala	45	46	45
T. chebula	48	49	47
CHX	25	23	27
Amoxicillin	20	18	21
DMSO	0	0	0

The data were analysed by Oneway Anova followed by Tukey – HSD (Post-Hoc) multiple comparison test and Kruskal-Wallis test.

Table 1.2 - ANOVA

	Mean	SD
Triphala	45.33	0.577
T. chebula	48	1
CHX	25	2
Amoxicillin	19.67	1.528
DMSO	0	0

Table 1.3 - TUKEY – HSD MULTIPLE COMPARISONS

Comparisons	Mean Difference	p value
Triphala vs T. chebula	-2.667	> 0.05
Triphala vs CHX	20.333	< 0.001
Triphala vs AMOX	25.667	< 0.001
Triphala vs DMSO	45.333	< 0.001
T. chebula vs CHX	23.000	< 0.001
T. chebula vs AMOX	28.333	< 0.001
T. chebula vs DMSO	48.000	< 0.001
CHX vs AMOX	5.333	< 0.01
CHX vs DMSO	25.000	< 0.001
AMOX vs DMSO	19.667	< 0.001

Table 1.4 - KURSKAL – WALLIS TEST

	Mean Rank	p value
Triphala	11	< 0.01
T. chebula	14	
CHX	8	
Amoxicillin	5	
DMSO	2	

INFERENCE

- All the antimicrobial agents showed high statistically significant difference ($p < 0.001$) when compared with DMSO.
- There was no statistically significant difference between Triphala and T. chebula ($p > 0.05$)

- Triphala and T. chebula showed high significant difference ($p < 0.001$) when compared with Amoxicillin and CHX.
- CHX showed significant difference ($p < 0.01$) when compared with Amoxicillin.

II. The **OD Values** measured for all the groups at **200 µm & 400 µm** are presented in the Table 2.1 & 2.2.

Table 2.1 – OD Values (200 µm)

Group 1	0.474	0.482	0.486	0.461	0.419	0.456
Group 2	0.003	0	0.001	0.002	0	0
Group 3	0	0.002	0.002	0.001	0	0.001
Group 4	0	0.001	0.002	0.001	0.001	0.003
Group 5	0.001	0	0.002	0.001	-	-

Table 2.2 – OD Values (400 µm)

Group 1	0.468	0.462	0.473	0.458	0.42	0.459
Group 2	0	0.002	0	0.001	0.003	0
Group 3	0.002	0	0.001	0	0.001	0.001
Group 4	0.001	0.028	0.011	0.026	0.042	0.018
Group 5	0	0.001	0	0.002	-	-

The data were statistically analysed using Oneway Anova followed by Tukey – HSD (Post-Hoc) multiple comparison test and Kruskal-Wallis test was done for non-parametric data.

Table 2.3 – ANOVA (OD Values)

Groups	200 µm		400 µm	
	Mean	SD	Mean	SD
Group 1	0.46300	0.024495	0.45667	0.018843
Group 2	0.00100	0.001265	0.00100	0.001265
Group 3	0.00100	0.000894	0.00083	0.000753
Group 4	0.00133	0.001033	0.02100	0.014311
Group 5	0.00100	0.000816	0.00075	0.000957

Table 2.4 - TUKEY – HSD MULTIPLE COMPARISONS

Goups Comparison	200 µm		400 µm	
	Mean Difference	p value	Mean Difference	p value
G1 vs G2	0.462000	< 0.001	0.455667	< 0.001
G1 vs G3	0.462000	< 0.001	0.455833	< 0.001
G1 vs G4	0.461667	< 0.001	0.435667	< 0.001
G1 vs G5	0.462000	< 0.001	0.455917	< 0.001
G2 vs G3	0.000000	> 0.05	0.000167	> 0.05
G2 vs G4	-0.000333	> 0.05	-0.020000	< 0.05
G2 vs G5	0.000000	> 0.05	0.000250	> 0.05
G3 vs G4	-0.000333	> 0.05	-0.020167	< 0.05
G3 vs G5	0.000000	> 0.05	0.000083	> 0.05
G4 vs G5	0.000333	> 0.05	0.020250	> 0.05

Table 2.5 – KRUSKAL – WALLIS TEST (OD Values)

Goups	200 µm		400 µm	
	Mean rank	p value	Mean rank	p value
Group 1	25.50	< 0.01	25.50	< 0.001
Group 2	10.50		9.08	
Group 3	11.17		9.08	
Group 4	13.00		18.42	
Group 5	11.25		8.38	

INFERENCE

At 200µm:

- Group 1 showed highly significant difference ($p < 0.001$) when compared with all other groups.
- Group 2, 3, 4 & 5 did not show any significant difference ($p > 0.05$) among them at 200µm.

At 400µm:

- Group 1 showed highly significant difference ($p < 0.001$) when compared with all other groups.
- Group 2 & 3 did not show any statistically significant difference ($p > 0.05$) between them and when compared with Group 5.

- Group 2 & 3 had statistically significant difference ($p < 0.05$) when compared with Group 4.

III. The **CFU / ml values** measured for all the groups at **200 µm & 400 µm** are presented in the Table 3.1 & 3.2.

Table 3.1 – CFU / ml Values (200 µm)

Group 1 (x 10^9)	156	168	170	148	142	154
Group 2	0	0	0	0	0	0
Group 3	0	0	0	0	0	0
Group 4	0	0	0	0	0	0
Group 5	0	0	0	0	-	-

Table 3.2 – CFU / ml Values (400 µm)

Group 1 (x 10^9)	148	152	164	152	150	160
Group 2	0	0	0	0	0	0
Group 3	0	0	0	0	0	0
Group 4	0	10	0	10	20	0
Group 5	0	0	0	0	-	-

Table 3.3 - ANOVA (CFU / ml)

Groups	200 µm		400 µm	
	Mean	SD	Mean	SD
Group 1 (x 10^9)	156.33	10.985	154.33	6.250
Group 2	0.00	0.000	0.00	0.000
Group 3	0.00	0.000	0.00	0.000
Group 4	0.00	0.000	6.67	8.165
Group 5	0.00	0.000	0.00	0.000

Table 3.4 - TUKEY – HSD MULTIPLE COMPARISONS

Groups Comparison	200 µm		400 µm	
	Mean Difference	p value	Mean Difference	p value
G1 vs G2	156.333 x 10^9	< 0.001	154.333 x 10^9	< 0.001
G1 vs G3	156.333 x 10^9	< 0.001	154.333 x 10^9	< 0.001
G1 vs G4	156.333 x 10^9	< 0.001	147.667 x 10^9	< 0.001
G1 vs G5	156.333 x 10^9	< 0.001	154.333 x 10^9	< 0.001
G2 vs G3	0.000	> 0.05	0.000	> 0.05
G2 vs G4	0.000	> 0.05	-6.667	> 0.05
G2 vs G5	0.000	> 0.05	0.000	> 0.05
G3 vs G4	0.000	> 0.05	-6.667	> 0.05
G3 vs G5	0.000	> 0.05	0.000	> 0.05
G4 vs G5	0.000	> 0.05	6.667	> 0.05

Table 3.5 – KRUSKAL – WALLIS TEST (CFU / ml Values)

Groups	200 µm		400 µm	
	Mean rank	p value	Mean rank	p value
Group 1	25.50	< 0.001	25.50	< 0.001
Group 2	11.50		10.00	
Group 3	11.50		10.00	
Group 4	11.50		15.50	
Group 5	11.50		10.00	

INFERENCE

At 200 µm & 400 µm:

- Group 1 showed highly significant difference ($p < 0.001$) when compared with all the other groups.
- Group 2, 3, 4 & 5 didn't have any statistical significance ($p > 0.05$) among them.

IV. **OD values and CFU / ml** at **200 µm & 400 µm** for each individual group were compared using independent sample test (Student t-test).

Table 4.1 – Independent Samples Test (Student t-Test)

Groups		CFU / ml			OD		
		Mean	SD	p value	Mean	SD	p value
Group 1	200µm	156.33×10^9	10.98484×10^9		0.46300×10^9	0.0245×10^9	
	400µm	154.33×10^9	6.25033×10^9	> 0.05	0.45670×10^9	0.0188×10^9	> 0.05
Group 2	200µm	0	0		0.00100	0.0013	
	400µm	0	0	> 0.05	0.00100	0.0013	> 0.05
Group 3	200µm	0	0		0.00100	0.0009	
	400µm	0	0	> 0.05	0.00083	0.0008	> 0.05
Group 4	200µm	0	0		0.00133	0.0013	
	400µm	6.6667	8.16497	> 0.05	0.02100	0.0143	< 0.05
Group 5	200µm	0	0		0.00100	0.0008	
	400µm	0	0	> 0.05	0.00075	0.001	> 0.05

Table 4.2 – Mann-Whitney Test

	p value	
Group 4	CFU	OD
	200 & 400	200 & 400
	> 0.05	< 0.05

INFERENCE

- There was no statistically significant difference ($p > 0.05$) between 200µm & 400µm values, when assessed by OD or CFU / ml in Group 1, 2, 3 & 5.
- In Group 4, there was no statistical significance ($p > 0.05$) between the 200 µm & 400 µm values, when assessed by CFU / ml. But, there was statistically significant difference ($p < 0.05$) when OD values were analysed.

INFERENCE OF THE STUDY

I Zone of Inhibition:

- All the antimicrobial agents showed high statistically significant difference ($p < 0.001$) when compared with DMSO.
- There was no statistically significant difference between Triphala and T. chebula ($p > 0.05$)
- Triphala and T. chebula showed high significant difference ($p < 0.001$) when compared with Amoxicillin and CHX.
- CHX showed significant difference ($p < 0.01$) when compared with Amoxicillin.

II OD Values:

- Group 1 showed highly significant difference ($p < 0.001$) when compared with all other groups at 200 & 400 µm.
- Group 2, 3, 4 & 5 did not show any significant difference ($p > 0.05$) among them at 200µm.
- Group 2 & 3 did not show any statistically significant difference ($p > 0.05$) between them and when compared with Group 5.
- Group 2 & 3 had statistically significant difference ($p < 0.05$) when compared with Group 4.

- In groups 1, 2, 3 & 5 there was no statistically significant difference ($p > 0.05$) between 200 & 400 µm values, within a group, when assessed by OD values.
- There was statistically significant difference ($p < 0.05$) between 200 & 400 µm values in Group 4.

III CFU/ml Values:

- Group 1 showed highly significant difference ($p < 0.001$) when compared with all the other groups.
- Group 2, 3, 4 & 5 didn't have any statistical significance ($p > 0.05$) among them.
- In all the groups there was no statistically significant difference between 200 µm & 400 µm values, within a group, when assessed by CFU/ml.

DISCUSSION

Microorganisms play a fundamental role in the etiology of pulp and periapical diseases. Their control and elimination are important during endodontic treatment.[47] E. faecalis is probably the species that can best adapt to and tolerate the ecologically demanding conditions in the filled root canals. It is resistant to various antimicrobials. It is considered as a 'star' in post-treatment disease.[40] Because of this the development of effective therapeutic modalities against E. faecalis is desirable. Hence E. faecalis was chosen for the inoculum in this study. In addition, E. faecalis is relatively easy to culture and it has been used successfully in most studies with the original model.[5, 37, 50, 52, 66]

Various modalities are attempted to eliminate residual bacteria from the root canal, which includes high end technologies as with LASERS, PAD and ANILAD. Even though these show promising results, its applicability to all clinical settings and private practice may be difficult because of the exorbitant cost involved. A cost effective way to deal with the same situation is using intracanal medicaments. The use of an intracanal medicament helps in the elimination of bacteria that remains even after cleaning and shaping, there by providing an environment conducive for periapical tissue repair.[16]

Many medicaments were used as intracanal dressings in the past decades and according to their chemical basis, generally fall into the following categories: phenolic derivatives (eugenol, camphorated para-monochlorophenol, camphorated phenol, metacresyl acetate, beechwood creosote), aldehydes (formocresol), halides (iodine–potassium iodide), calcium hydroxide, antibiotics, and various combinations. The most popular intracanal medicament in use currently is calcium hydroxide. However, various studies confirmed the resistance of E. faecalis to Calcium hydroxide. Various newer medicaments are also tried nowadays, as Bioactive glass and Octanidine. 2% CHX has been found to be highly active against E. faecalis.[21, 44] CHX seems to act by adsorbing on to the cell wall of microorganisms and causing the leakage of intracellular components. It also has the property of substantivity.[5, 37, 50, 52, 96]

There has been a worldwide move towards the use of traditional medicines due to concerns over the more invasive, expensive and potentially toxic mainstream practices (WHO, 2002). Numerous studies have identified compounds within herbals that are effective antibiotics (Basile *et al.*, 2000; Cowan, 1999). Traditional healing systems around the world that utilize herbal remedies are an important resource for the discovery of new antibiotics (Okpekon *et al.*, 2004). Certain traditional remedies have already been reported to be effective against drug-resistant bacteria (Kone *et al.*, 2004; Sato *et al.*, 2000). These facts provoked us to

test an herbal preparation as an intracanal medicament, to find the activity against E. faecalis.

Triphala (a Siddha Herbal Formulation) and T. chebula were selected because of their known broad spectrum of antimicrobial activity. Triphala has been used extensively as a drug against a number of diseases (Awasthi and Nath, 1986; Reddy et al., 1990). Triphala is prescribed for various symptoms of infections, fatigue, assimilation and infectious diseases such as tuberculosis, pneumonia, AIDS (El-Mekkawey and Merelhy, 1995), periodontal diseases (Abraham et al., 2005) and has been reported to reduce considerably the damage due to oxidative stresses in experimental rats (Srikumar et al., 2006). Because of its tremendous potential, it is called the 'Wonder Drug'.

T. chebula is reported to have antioxidant and free radical scavenging activities. It is effective against cancer cells[80] and various microorganisms.[56, 87] It is also useful as an anti-caries agent[87], in dermal wound healing[41], improving gastrointestinal motility, anaphylactic shock[84] and in diabetes mellitus.[79] So, it is called the 'King of Medicines'.[14]

Alcoholic extracts of Triphala and T. chebula were selected as they showed high antibacterial activity compared with aqueous extracts, which might possibly be due to the lesser solubility of the active components in

aqueous solutions.[87] DMSO was used as a solvent, as it does not have any antibacterial action on its own,[56] which had been proved in previous studies and in this study by using it as a control. It is a highly polar organic reagent that has exceptional solvent properties for organic and inorganic chemicals. Thus, it helps in complete dissolution of the extracts.

Assessment of MIC was done to find the concentration of the extracts needed to show antibacterial activity. In this study, MIC was found to be 20mg / ml for Triphala and 30mg / ml for T. chebula. But in a previous study, the MIC for Triphala and T. chebula against E. faecalis were found to be as low as 0.1 µg / ml.[87] This could be because of the effects of geographic variations on the phytochemical concentratation.[42]

The agar diffusion method has been widely used to test the antimicrobial activity of dental materials and medicaments.[31] So, this was used to compare the zone of inhibition produced by the antimicrobial agents used in this study. Among them, Triphala & T. chebula showed highly significant difference and proved to be superior when compared with CHX & Amoxicillin. CHX showed superior antimicrobial action when compared with Amoxicillin. There was no statistically significant difference between Triphala & T. chebula. This revealed that, the extracts have superior antimicrobial activity against E. faecalis. The advantage of this method is that, it allows direct comparison of the materials against

the organisms, indicating which material has the potential to eliminate bacteria in the local microenvironment of the root canal system. However, the limitation of this method is that the result not only depends on the toxicity of the material for the particular organism, but also is influenced by the ability of the material to diffuse across the medium.[74] Further, the materials which show antimicrobial activity in culture plates may not have the same when used as an intracanal medicament, because of the intricacies of the canal system and inherent ability of the dentin to have a negative impact on medication performance.[59]

Generally, bacteria are present in a state of biofilm in the root canals. Estimate of 1000 to 1500 times greater resistance for biofilm grown cells than planktonically grown cells have been suggested. This may turn the same medicament which showed activity against planktonic bacteria to be inactive in clinical scenario. The nature of the substrate may also influence the nature of the biofilm. So, we opted to test the activity of the herbal extracts against the biofilm produced in teeth samples which may also help in assessing the activity in different dentin penetration depths.

The experimental model used in this study was adapted from that established by Ørstavik and Haapasalo for infection and disinfection of dentinal tubules. The model was adapted for extracted human teeth rather than bovine incisors. This modification was considered appropriate

because of the marked difference in diameter between the canals of bovine and human teeth and thus in the volume of medicament that can be placed in these canals. Propylene glycol was used as a vehicle for the herbal extracts, as it can deliver the medicaments rapidly and more effectively in the root canal system and within dentinal tubules.[18]

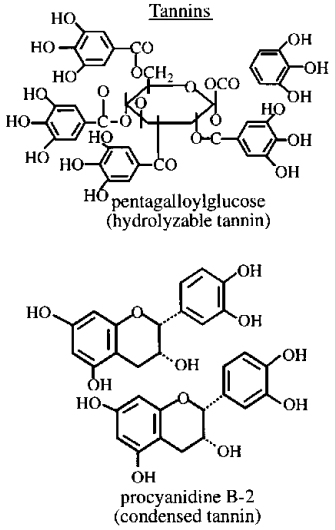
In this study all the medicaments tested showed significant antimicrobial action on E. faecalis when compared with the control group, when assessed by the colony count and OD values. When CFU/ml were considered, Triphala and T. chebula showed 100% inhibition at both 200 µm and 400 µm in all the samples. 2% CHX showed 100% inhibition in all the samples at 200 µm depth and 50% of the samples showed 100% inhibition at 400 µm. There was statistical significance between the test groups and the positive control group. But, there was no statistically significant difference between CHX, Triphala and T. chebula.

When OD values were considered, all the groups showed significant difference with the positive control group. There was no significant difference between CHX, Triphala and T. chebula at 200 µm depth. But, there was statistically significant difference between the herbal extracts and CHX at 400 µm depth. Even though there is no statistically significant difference between test groups when CFU/ml were considered, complete inhibition of the organism at both the depths in all

the Triphala and T. chebula treated specimens may have some clinical significance.

Tannic acid represents the major constituent of the ripe fruit of T. chebula and is present in a concentration of 20–40% (Chopra and Handa, 1958). The chief constituents of tannin are chebulic acid, chebulagic acid, corilagin and gallic acid. Tannin of Terminalia chebula are of pyrogallol (hydrolyzable) type. A group of researchers found 14 components of hydrolyzable tannins (gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulegic acid, chebulinic acid, 1,2,3,4,6- penta-O-galloyl-H-D-glucose, 1,6,-di-O-galloyl-D-glucose, casuarinin, 3,4,6-tri-O-galloyl-D- glucose, terchebulin) from Terminalia chebula fruits.[14] The fruits also contain a variety of carbohydrates, glucose and sorbitol being the major ones. The pericarp of the fruit contains anthraquinone glycosides, saponins, anthrones and anthranols.

Model structure of Tannins



The dried fruits of T. bellerica contain about 20% tannins of both condensed and hydrolysable type. Other constituents identified in the fruit include lipids, b-sitosterol, saponins, gallic and ellagic acids and their derivatives, glycosides and various carbohydrates.

Phyllanthus emblica also contains hydrolysable tannins (emblicanin A, emblicanin b, punigluconin 12% and pedunculagin).

The total tannin content of the extracts used in this study was found to be 42.25% w/w for Triphala and 42.80% w/w for T. chebula. The antibacterial activities of tannins are well documented. Tannins inhibited the growth of many fungi, yeasts, bacteria and viruses. Tannins in these fruits thus serve as natural defense mechanisms against microbial infections.[14]

One of their molecular actions is to complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, their mode of antimicrobial action, may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc.[56] Because of this the antibacterial activity could be attributed to either inhibiting the cell division or to damaging the cell walls of bacteria.[87] They also complex

with polysaccharide. The antimicrobial significance of this particular activity was not explored earlier.[56] We speculate that this property of tannins may aid in complexing with glycocalyx (Extracellular polymeric substance which contains polysaccharides and surrounds the microcolonies and anchor the bacterial cell to the substrate) and disrupt biofilm, which may greatly aid in enhancing the antimicrobial action over biofilm. From the literature it has been noted that T. chebula exhibited significant hepatoprotective, cardioprotective, antimutagenic/anticarcinogenic,[80] cytoprotective, radioprotective, antioxidant[63] and adaptogenic[76, 84] effects indicating that it is a safe substance to be used as a drug.[14]

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Further exploration should be done on these herbs, which can enable us to utilize the gift of divine nature.

SUMMARY

Endodontic treatment is targeted towards reduction and elimination of microorganisms from the root canal system. Intracanal medicaments can aid in achieving this objective. This study evaluates the antimicrobial activity of Triphala & T. chebula against E. faecalis, in comparison with 2% CHX. Minimal inhibitory concentration of the alcoholic extracts of Triphala & T. chebula was found using agar-dilution method. Agar diffusion method was used to compare the zone of inhibition produced by the herbal extracts & CHX. All the three showed significant antimicrobial activity when compared with the control groups. The herbal extracts showed significant difference when compared with CHX.

Activity on biofilms formed in teeth samples were studied using a modification of the model proposed by Haapasalo & Orstavik. The dentin blocks were infected with E. faecalis for 21 days, then they were divided into 4 groups based on the medication given, as

Group I – Saline (Infected dentine blocks),

Group II – Triphala,

Group III – T. chebula &

Group IV – CHX.

The medicaments were maintained for 7 days. Uninfected dentine samples served as negative control in Group V. At the end of 1 week, antimicrobial efficiency was assessed at the depths of 200µm & 400µm, by colony count (CFU / ml) & optical density (OD) values. All the 3 medicaments showed statistically significant antimicrobial activity. Assessment of the antimicrobial activity using OD values did not show any statistically significant difference among the test groups at 200µm. But there was statistically significant difference between the herbal extracts and CHX at 400µm.

On microbial culturing, Triphala & T. chebula showed 100% inhibition of growth of bacteria in all the samples. CHX showed 100% inhibition in all the samples at 200µm and in 50% of the samples at 400µm. Assessment of the colony count (CFU / ml) showed significant antimicrobial activity for all of the medicaments. The difference shown between the extracts & CHX in the colony count was not statistically significant. But complete inhibition shown by the herbal extracts in all the samples may have some clinical significance. The results showed the potential of Triphala and T. chebula to be used as intracanal medicament.

CONCLUSION

Results of this study indicate that E. faecalis biofilm present in the root canal system can be eliminated or reduced with intracanal medicaments. The herbal extracts of Triphala, T. chebula have the potential to be used as intracanal medicaments, as the already established 2% of CHX. They also have antimicrobial action on the E. faecalis biofilm penetrated within the dentinal tubules. The herbal extracts may have superior antimicrobial activity even at deeper dentine depths. Thus, this study opens a new avenue of trying herbal extracts as intracanal medicaments to eliminate the microorganism in the root canal system and aid in long term success in endodontics.

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